A STUDY OF RHABDOMYOSARCOMAS INDUCED BY NICKEL SULPHIDE IN RATS

by

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ABSTRACT

The study of rhabdomyosarcomas induced by nickel sulphide was undertaken in order to investigate their growth and responsiveness characteristics and to evaluate their possible usefulness as experimental tumour systems. A study was also made on the carcinogenic specificity of nickel sulphide.

Intramuscular injections of 0.5 to 20 mg. of Ni_3S_2 resulted in tumours after two to six months, which grew fairly rapidly, once palpable. On transplantation they proved to be highly malignant, causing 100 per cent takes within approximately two weeks and death of the host within three to eight weeks. Detailed histological study was performed on many sections of tumour and muscle in order to establish identity of the rhabdomyosarcomas and to observe such cytological phenomena as muscle degeneration, tissue disruption and development of anaplasia.

The carcinogenic activity of Ni_3S_2 was emphasized by the rapidity of tumour induction observed after very low doses, while the difference in latent periods suggested some correlation between dose and response. Injections of Ni_3S_2 into body organs illustrated the extreme toxicity of the substance in individual tissues. Although results were somewhat inconclusive within the time limit of the experiment, the

general tenor of the data suggested that Ni_3S_2 had a toxic rather than tumourigenic action on most tissues. The appearance of rhabdomyosarcomas when the compound was in contact with abdominal or leg musculature gave evidence that nickel sulphide might have some specificity for striated muscle. Preliminary experiments with NiS indicated that its carcinogenicity, if any, was considerably less than that of Ni_3S_2, with different solubility in muscle being a possible explanation.

Several of the induced tumours were used for metabolic and experimental therapy studies with various compounds. Numerous experiments indicated that the tumour was essentially unreliable for such studies since growth rates and response were not reproducible in different tumour generations. However, some general trends were noted. Response to the corticoids ranged from residual to complete inhibition of many tumours during the period of treatment with cortisol or cortisone. Subcutaneous injections of testosterone showed acceleration of growth in some tumour lines. Tests with the Vinca alkaloids suggested some correlation with clinical anti-tumour effects, indicating an increased effectiveness of both Vinblastine and Vincristine when administered during the first week after transplantation.

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INTRODUCTION

Metal Carcinogenesis

In 1959 Hueper presented a comprehensive review of the known occupational carcinogens, which number over two hundred, and stated that several metals, including arsenic, chromate, beryllium and nickel, exhibited potential causal relationship to cancer in humans (50). He emphasized the potency of the suspected carcinogens by stating that if a person were exposed to a carcinogenic agent the ensuing development of cancer was only a matter of time. The time lag between exposure and tumour appearance, or the latent period, has been shown to be characteristically long in metal carcinogenesis, with the average latent period for arsenical cancer being twenty-five years; for chromate, fifteen years; for nickel, twenty-two years. These figures served to re-emphasize the main characteristic of environmental and occupational cancers which had been implied by Potts almost two hundred years ago, when he presented his classical account of scrotal cancer in chimney sweeps (41). He was the first to recognize the long latent period between exposure and development of the disease. As well as time for development, the other determining factor in metal carcinogenesis was said to be the

amount of exposure. Most of the proven carcinogenic metals exerted their deleterious effects after inhalation, as had been indicated in the many reports of lung and nasal cancers. It was therefore suggested by Hueper that a man in a refinery who worked harder at his job and breathed more deeply could have a greater chance of getting lung cancer, if a harmful metal were present in the dust, than someone on a less physically strenuous job in the same exposed location (50).

Neubauer has stated that the earliest report of cancer linked to a specific metal appeared in 1822 when Paris reported cases of skin cancers in men working in copper smelters and tin refineries (65). His belief that the neoplasms were due to arsenic in the process fumes was generally ignored for many years. In 1879 some cases of lung cancer in Germany were attributed to the presence of arsenic in the dust of certain factories. This evidence still was not accepted, since it was felt that those afflicted had been exposed to other carcinogenic agents. Since that time, however, the implication of arsenic as a causal agent has gradually become better established. Although the number of cases of lung cancer reported has not been great, there seemed to be significantly more deaths from lung cancer among arsenic-exposed workers than among the average

population (43). Arsenic has also been shown to cause skin cancer when ingested (65). Animal experiments with arsenic have not been conclusive, since the latent period required was generally longer than the life span of the animals.

A similar struggle ensued to have chromate recognized as a possible metal carcinogen. From 1911 to 1932 there were occasional reports of respiratory system tumours associated with exposure to chromates. The first lung cancer positively linked with chromate was in 1911 in Germany, when two cases were reported by Pfeil (7). Twenty-four years later he listed another five cases in the same plant. Machle reviewed the relationship of chromates to cancer in the United States and stated that between 1937 and 1947, forty-two cancer deaths, most of them due to bronchogenic carcinomas, had been reported in men working in the chromium industry (60). With Switzerland and England contributing only occasional cases, Germany and the United States recorded a majority of the 122 cases of respiratory tract cancer reported in chromate workers up to 1950 (7). The exposure time was between sixteen and twenty-two years, and the average age of death was fifty-two years. The death rate for lung cancer in chromate workers in the United States was calculated to be twenty-five times greater than the normal rate (60) and the carcinogenicity of chromate has now been acknowledged.

Beryllium is one of the metals which as yet has been considered only as a possible carcinogenic hazard to man. Clinically, inhalation of beryllium oxide has led to acute and chronic pulmonary inflammations, but not to neoplastic changes. Experimentally, however, osteogenic sarcomas have been produced in rabbits either by intravenous injection or by inhalation of dust containing beryllium oxide (22). The latter method is particularly interesting since it suggests that the beryllium particles might be phagocytosed in passage through the lung and carried via blood and lymph to bone, a process which could conceivably occur in man.

Experimental studies of the possible carcinogenic effects of metals have assumed importance in view of their extensive use not only in industry but also in common commercial products. Arsenic is found in ores of copper, zinc, silver, cobalt and lead, and is employed as a pesticide and in weed-killers. Chromate is used in stainless steel, inks and paints, as well as in the manufacture of gas turbines and jet engines. Beryllium has been incorporated into electrical heating elements(50).

The emergence of nickel as a carcinogenic compound was recorded in 1932 when lung and sinus cancers were noted among men in the Mond nickel works (22). Hueper reported that in 1949 the annual report of the Chief Inspector of Factories and

Workshops in Britain listed forty-seven cases of nasal cancer and eighty-two of lung cancer in England during the period 1923 to 1948 (47). Doll estimated that this incidence was thirty times above normal for nasal cancer and five times above normal for lung cancer, implying a definite carcinogenic agent somewhere in the nickel refineries (19). As with some of the other metals mentioned, the latent period did not depend on the initial age of exposure, but on the actual length of the exposure period. Passey presented a theory that since there was often a long preceding history of irritation or lung damage such as bronchitis, there may have been excess mucus blanketing normal exchange of gases and fluids underneath, causing nickel to settle into the alveoli (69). A similar hypothesis was suggested by Hueper, who felt that inhaled nickel carbonyl could be rapidly decomposed in the lungs to carbon monoxide and metallic nickel, the latter then being precipitated on respiratory surfaces (49).

This circumstantial evidence of the carcinogenic activity of nickel compounds presents an interesting problem in view of the ubiquitous occurrence of nickel and its salts in everyday circumstances. Nickel has been found generally in soils and plants throughout the world. The largest deposits for industrial purposes are found in Canada and New Caledonia,

where it occurs as deposits of sulphide ore. Nickel is known as a particularly common component of industrial processes and household articles. Industrially it is used in preparing alloys with copper, magnewium, zinc, chromium, iron and molybdenum, and in making corrosion-resistant and heat-resistant steel and cast iron (57). Two processes which have developed the most widespread industrial use of nickel are electroplating, in which nickel ammonium sulphate is used as the electrolytic fluid, and hydrogenation of oils, in which pure nickel serves as a catalyst (23). Some of the more common articles containing nickel include stainless steel products, kitchen utensils, linoleum, paints, inks, oil, storage batteries, coins and jewelry (47, 48, 57).

Hueper has performed several experimental studies on the carcinogenic activity of nickel which was obtained from industrial disintegration of nickel carbonyl. Interest arose because of circumstantial evidence involving adverse effects of the latter compound. Injection of fifty milligrams of metallic nickel into femoral and pleural areas resulted in 30 per cent tumours in seven to sixteen months. In some lungs nickel was embedded in pleura and capillaries, indicating that it could be precipitated there. Of the femoral area tumours only 25 per cent were of muscular origin (48). Other tumour

types included osteogenic spindle cell sarcomas, squamous cell carcinomas and reticulum cell sarcomas, while an earlier experiment had shown no muscle tumours at all (46). He concluded, from the variety of histological neoplasms present, that although nickel did have carcinogenic activity, there was no tissue specificity associated with nickel carcinogenesis. This point of view was in direct contrast to that of Gilman who felt that, experimentally, nickel showed a definite preference for striated muscle. Both observations are subject to some criticism with regard to the experimental work. Gilman injected nickel mainly into areas of striated muscle, with only a few animals receiving nickel in other body areas (30). The latter experiments consisted of injections into the abdominal cavity which resulted in a few tumours involving the omentum and diaphragm. Hueper, on the other hand, obtained the majority of his data from experiments involving injections into the bone marrow of the femur, where it is conceivable that leakage along the injection path could have resulted in diverse tumours of the surrounding tissues.

Rhabdomyosarcomas

Primary tumours developing from the mesenchymal element of muscle are considered to be extremely rare. The most

malignant type is known as a rhabdomyosarcoma or tumour of striated muscle. Clinically it is stated that these neoplasms make up ten to fifteen per cent of sarcomas of the soft somatic tissues, and that approximately two-thirds arise in the lower extremities. Other gross characteristics have been described such as limited mobility, rapid growth and invasiveness, necessitating extensive removal of tissue or complete limb amputation, if feasible (1).

The spontaneous appearance of rhabdomyosarcomas in animals has occurred only rarely. Isolated reports of such tumours have appeared since 1896, describing neoplasms in the tail of a stallion, the kidney of a hog, the heart of a cow, and in trout and codfish (10). The earliest documented report of the neoplasm in rodents was in 1922 when Bullock and Curtis described a chondrorhabdomyosarcoma in the sternal musculature of a rat (10). The tumour had the now familiar appearance of giant cells and irregular polyhedral cells with cross-striations. Maddock, Kury and Riley reported another spontaneous rhabdomyosarcoma in 1962, this one occuring in the sternal area as well (61). Its original appearance, highly fibrous with small round cells, eventually changed to one of complete pleomorphism, with no relationship to the striated fibers of origin.

Experimental production of rhabdomyosarcomas has been equally uncommon. In 1954 Heath began testing the carcinogenic action of cobalt (36). Injection of approximately twenty-eight milligrams of pure cobalt into Hooded rats resulted in the induction of 50 per cent rhabdomyosarcomas after six months. Further studies on the activity of injected cobalt in muscle led to the suggestion that this metal seemed to have some specificity for causing striated muscle tumours (37).

Another report of their experimental induction by metals followed from Gilman and colleagues at Guelph, who announced that rhabdomyosarcomas could be induced in certain strains of experimental animals by nickel sulphide (29). Work had begun in 1960 on testing the effects of injection of a refinery dust which contained nickel, cobalt, copper and iron silicon, in order to investigate the possibility that such dust might be carcinogenic (28). Injection of this mixture intramuscularly into rats and mice resulted in approximately 70 per cent tumours arising at the site of injection. The latent period in mice was twice as long as in rats and the tumours were mainly fibrosarcomas, while those in the latter species were rhabdomyosarcomas.

As an extension of this experiment a study on the carcinogenic activity of various salts of the heavy metals

mentioned previously was performed (29). A standard dose of twenty milligrams per thigh muscle was administered to rats. The response of Hooded and Wistar rats to cupric oxide, ferric oxide and nickel sulphate was negative after 415 days, while nickel oxide, cobalt oxide and nickel sulphide (Ni_3S_2) showed a positive response. The latter compound produced the greatest number of tumours.

Almost all of the tumours induced by nickel sulphide and cobalt oxide, and a majority of those induced by nickel oxide, were rhabdomyosarcomas, highly cellular and pleomorphic. Metastases to both lungs and lymph nodes were noted in 95 per cent of the rats given nickel sulphide. In an attempt to correlate tumour incidence with dosage of nickel sulphide, it was found that occasional tumours (3 per cent) appeared in rats given intramuscular doses as low as 0.5 milligrams, with some relationship being shown between size of initial dose and per cent response. The conclusion was made that the observed carcinogenicity of certain nickel and cobalt compounds supported the hypothesis that such dusts in the refinery industry might constitute an industrial cancer hazard. This would tend to substantiate the evidence obtained in refinery studies, where the majority of tumours have involved lung and nasal areas which

would logically be exposed to the dust. Further interest has since been concentrated on nickel sulphide as a means of inducing rhabdomyosarcomas since it appeared to be the most potent metal compound of those tested.

Present Investigation

The use of nickel sulphide as an agent for the inducement of rhabdomyosarcomas in animals was originally adopted for the present investigation in an attempt to obtain a reliable tumour system for experimental testing of a group of chemotherapeutic compounds known as Vinca alkaloids.

Relatively few cases of treatment of these neoplasms by chemotherapeutic agents have been cited in clinical literature. However, a recent article by Whitelaw and Teasdale reported treatment of a rhabdomyosarcoma with an alkaloid used in treatment of other solid tumours (83). A six-month old boy was examined for a lump on his buttock, which was diagnosed as an embryonal rhabdomyosarcoma. Radiation had no effect on the tumour mass and by eight months it had spread to the right inguinal area and the right lower abdomen. The compound selected for chemotherapy, Vinblastine, was administered twice in doses of 1.5 milligrams, injected intravenously. The tumour regressed, then recurred again at twelve months. At this time two doses of

2.5 milligrams were administered and repeated again in two weeks. After this treatment, the tumour disappeared completely and showed no sign of recurrence at sixteen months. Vinblastine has been tested against a large number of human tumours which have exhibited varied responses. Most effective response has been obtained in Hodgkin's disease, cancer of the breast and lung, and chorioepithelioma. In an extensive review of the Vinca alkaloids, Johnson et al cited four cases of rhabdomyosarcomas which had been treated with Vinblastine(57). Clinically the dose has been somewhat limited by a leukopenic effect which may cause a generalized increased susceptibility to infection.

The Vinca alkaloids are of interest to this Research Centre since they have been used extensively in experimental tumour studies. Although clinical introduction of these compounds was not made until 1958, the original experimental observations were recorded in 1949 when Noble was studying the effects of an extract of the periwinkle plant, Vinca rosea (Linn.), on carbohydrate metabolism (69). Although primitive folklore had suggested its value in the control of diabetes mellitus, extracts of the plant given orally to experimental animals proved to have no effect on the blood sugar of normal

or diabetic rats or rabbits. In an attempt to increase the possible effectiveness of the extract it was administered intraperitoneally to rats. Again there was no change in the blood sugar level but an unexpected side-effect was observed. In most cases death occurred in five to seven days from a rapid, severe infection shown to be associated with a marked reduction in circulating leukocytes. Concomitant with the rapidly falling white blood count there was a marked destruction of granulocytes and profoundly depressed bone marrow. It was decided to use the leukopenic effect in rats as an assay method in attempting to isolate an active principle from crude extracts of periwinkle. Beer was eventually able to isolate a crystalline alkaloid from a number of active substances in the plant (17). It was subsequently shown to be of a new class of chemical compounds, dimeric indole alkaloids, and was called Vincaleukoblastine (Vinblastine). Concurrent research carried out at the Eli Lilly laboratories resulted in the isolation of a number of other alkaloids, one of which, Leurocristine (Vincristine), varied only slightly in chemical composition (51).

Both alkaloids have now been tested against various animal tumours such as the P1534 leukemia, Ehrlich ascites, chloroleukemia, AKR leukemia and others. In many cases the

anti-tumour effect has been limited by the leukopenia which results from higher doses. Although Vinblastine and Vincristine have both demonstrated anti-mitotic activity in all tumours tested, these results have not correlated with anti-tumour activity (51). The alkaloids appeared to effect the most striking inhibition of tumour growth or number of takes when administered soon after transplantation. Testing of the compounds against new tumour lines has been standardized by giving ten consecutive daily injections, beginning twenty-four hours after transplantation.

It was believed, therefore, that a reliable rat rhabdomyosarcoma might serve as an experimental model for Vinblastine and Vincristine, particularly in view of the observed clinical effects of the former on rhabdomyosarcomas in humans. Different dose regimens and times of administration were planned for chemotherapeutic testing on several lines of the induced tumours.

Because of the striated muscle origin of rhabdomyosarcomas they were considered to be useful for studying metabolic effects of certain anabolic and catabolic hormones, again with the idea of attempting to establish experimental models for various steroid hormones. In addition, the possible growth-

inhibiting effects of corticosteroids on this experimental neoplasm were of some therapeutic interest.

At the same time, the induction of such tumours for the above studies offered an opportunity to study a phase of metal carcinogenesis. An attempt was made to observe the specificity and general carcinogenic activity of nickel sulphide in rats. As well, the use of a reliable and potent agent for producing rhabdomyosarcomas was necessary in order to study their gradual development in muscle and other organs, as influenced by experimental variables such as dosage, sex, strain and age. More directly, it was desirable to observe growth characteristics and histological changes during the induction of rhabdomyosarcomas.

EXPERIMENTAL MATERIALS AND METHODS

Materials

Nickel Compounds. Nickel sulphide, in the form of Ni_3S_2, was prepared by International Nickel Company of Canada in the following manner (70):

"The fine nickel sulphide was produced by melting refined nickel metal with bright yellow sulphur. The molten sulphide was cast, solidified, crushed in a laboratory jaw crusher, ground in a disc pulverizer and the ground produced was further comminuted to the desired size in a laboratory pebble mill." Particle size was approximately five microns. The original sample was obtained from Dr. J.W. Gilman.

Nickel sulphide, in the form of NiS, was prepared by Dr. P.H. Jellinck at the Cancer Research Centre, U.B.C., in the following manner:

To twenty grams of nickelous chloride (Reagent Special) fifteen ml. of Analytical Reagent grade ammonium sulphide was added and the precipitate of nickelous sulphide filtered. The precipitate was repeatedly washed with warm distilled water until the filtrate was colourless and then washed successively with warm acetone, ethyl alcohol, benzene and acetone again. The powder was dried and pulverized.

Compounds were administered as suspensions in distilled water or sesame oil. Intramuscular injections were made into the gastrocnemius muscle of one leg. Injections into various organs were performed by surgical opening under ether anesthesia.

Vinca Alkaloids. Vincaleukoblastine and Leurocristine were supplied by Eli Lilly Company under the trade names of Vinblastine Sulphate (VLB) and Vincristine Sulphate (VCR) respectively. Both compounds were dissolved in physiological saline and injected intraperitoneally.

Hormones. Three corticosteroids, cortisol, cortisone and prednisolone, were obtained from Nutrient Biochemicals Corporation, and were administered intraperitoneally or subcutaneously in water. Testosterone Propionate was obtained from Fisher Chemical Company and was administered subcutaneously in sesame oil.

Methods

Experimental Animals. Male and female rats of the following strains were used: Hooded, Fischer, P.A., Wistar and Sprague-Dawley. The first three strains were from inbred stock maintained in the laboratory. All animals were maintained on Master's Fox Chow diet ad libitum and were examined at

periodic intervals for tumour formation. Tumour measurements were made with calipers in two dimensions and tumour weights were calculated from Shreck's formula, the third diameter being the mean of the two diameters measured (73).

Techniques of Transplantation. Routine transplantation was accomplished in the following manner. A small piece of tumour tissue was excised and chopped finely in a Petri dish. It was then homogenized in a glass blender with approximately equal volumes of physiological saline. Approximately ten million cells, determined by counting in a haemocytometer, were injected in 0.2 cc. of suspension. A #21 needle was used and injection was made into the gastrocnemius muscle of recipient rats. Tumours were assigned prefix numbers to denote new tumour lines and affix numbers to indicate transplant generations.

Techniques of Histology. Tissues were fixed in Bouin's fluid or 10% formal-saline. Paraffin sections were stained routinely with haematoxylin and eosin. Mallory's phospho-tungstic-acid haematoxylin and Perl's stains were applied to muscle, tumour and organ sections for specific histochemical studies. Basic techniques were obtained from Lillie's text-book of histology (59).

RESULTS

Tumour Induction Studies with Ni_3S_2

Injection into Muscle. Male and female Hooded rats weighing eighty to one hundred grams were injected intramuscularly with varying doses of Ni_3S_2, ranging from 0.5 to 20 mg. The animals exhibited no toxic side-effects or loss of weight after receiving the injections, and continued growing at a normal rate. Tumours were recorded as palpable when they were approximately one cm. in diameter or when an obvious swelling of the leg muscle was apparent. Animals were killed when tumours reached a size of three to four cm. in diameter, usually one to two months after the first appearance. At the time of death normal leg function was generally inhibited. Latent periods for tumour appearance ranged from ninety-two days for the highest dose to almost two hundred days for the lowest, with the first tumour appearing after fifty-eight days. After nine months animals which had received 10 and 20 mg. of Ni_3S_2 had 100 per cent tumours; those receiving 2.5 mg. had 66 per cent while those with 0.5 mg. had 40 per cent tumours. Results are summarized in Table I.

Autopsy showed that the tumours generally arose at the site of injection and were localized in the muscle region, replacing the gastrocnemius muscle which had degenerated. An

TABLE I

RESPONSE OF RATS TO INTRAMUSCULAR DOSES OF Ni_3S_2

Dose (mg.)	Number of animals	Tumours Number	Tumours %	Average latent period*
20	5	5	100	92 ± 24 days
10	3	3	100	171 ± 19 days
2.5	15	10	66	184 ± 21 days
0.5	15	6	40	191 ± 23 days

*first palpable as hard swelling within muscle

exception to this statement was seen in one female rat in which the tumour mass had infiltrated through the peritoneal wall and formed a large, continuous mass in the abdominal cavity. Particles of nickel were observable in most tumour masses, scattered thinly throughout the stroma. Most animals exhibited multiple nodules in the lung which were fairly hard and white in appearance. However, those examined histologically confirmed the gross observation of abcess formation. No other unusual nodules were found. Detailed histological examination of these tumours, to be presented later, corroborated gross evidence that they were highly malignant rhabdomyosarcomas. One tumour arising eleven months after injection demonstrated a different growth characteristic in that it was situated as a small white nodule within the normal muscle mass rather than in place of it, and it did not transplant. Histology of this tumour will be compared with those described above.

Female Wistar rats, eighty to one hundred grams, were injected with 20 mg. of Ni_3S_2 and exhibited four tumours among thirty animals. Many of the rats appeared to have swellings which arose during the third month at the site of injection. However, these all regressed within four weeks and no sign of any tumour formation was seen in these areas. Autopsy performed at random on several animals after ten months revealed that no

nickel particles were present in the injected muscle regions. Of the resulting tumours, only one was at the injection site, and it had spread from the muscle region into the lower right abdominal quadrant. This tumour, 7-N, was transplanted easily and grew rapidly, causing death to the host within three weeks. After eight months one rat developed a lump on the neck which did not take on subcutaneous transplantation, while another rat developed two tumours after sixteen months. These arose in the upper chest region and in the lower left groin; both were loosely palpable and attached to the skin. Histological detail is presented in another section.

Injection into Organs. In order to test the alleged specificity of nickel for inducing tumours in striated muscle, Ni_3S_2 was injected into various organs in Hooded rats, in doses of approximately 1 to 2 mg. Injections were made into liver, spleen, testis, prostate, uterus and ovarian capsule.

Two out of three animals receiving an intrahepatic dose developed huge tumours after six months which, at the time of death, filled the abdominal cavity and were grossly palpable from the external surface. Although part of the tumour was situated deep among the lobes of the liver, the origin appeared to be the peritoneal wall immediately overlying the liver. The

tumour had spread to the intestinal tract as well, where it appeared to be well attached. Transplantation of small pieces of the tumour was easily achieved for several generations. Both the original tumour and all transplants were typical rhabdomyosarcomas on histological examination. In another rat, the nickel was found to be enclosed in a small hard pocket of tissue adhered to the abdominal wall, but no tumour formation was seen.

The injection of Ni_3S_2 into testes did not appear to have deleterious effects on the general health of the rats. Autopsy performed after six and nine months showed that the nickel particles were still localized in the injection area. Grossly the testis contained black and gritty material, with the metal completely surrounding the seminiferous tubules. The untreated testis showed no abnormalities. Sections of these and other organs were made for histological study, to be described later.

Two rats which had received intraprostatic injections developed large lumps at the site shortly after the operation. Autopsy showed that this was abcess formation around the suture line. A third rat had a large swelling overlying the testicular area which was due to great displacement of the intestinal tract.

The prostate gland was hard and full of nickel, with no evidence of abnormal enlargement, while the testes did not seem to be affected by the intestinal compression.

A small white lump, approximately five mm. in diameter was located in the spleen of one rat which had received an injection in that area nine months previously. The nodule, which was very hard and showed some nickel particles in the center, occupied one end of the spleen and the rest of the organ appeared to be normal.

Injections into the ovarian capsule and the uterus produced no obvious changes in the organs when examined grossly after nine months.

In general the animals survived well. No deaths occurred as an immediate result of the operation and only two out of eighteen rats succumbed during the nine-month period.

Tumour Induction Studies with NiS

Injection into Muscle. For comparative purposes a second form of nickel sulphide, NiS, was tested for carcinogenic activity in rats. Intramuscular injections of NiS were made into a total of 150 rats in order to observe the effects of the following factors on tumour induction. Strain differences were tested by injecting NiS into female rats of six strains:

Hooded, Fischer, P.A., Sprague-Dawley, UBC. Wistar, Woodlyn Farm Wistar. Approximately half of the Sprague-Dawleys and UBC. Wistars died during the first two months after injection. The latter group was composed of younger rats, weighing thirty to forty grams, in contrast to average weights of eighty to one hundred grams in the other strains. After four months, none of the surviving rats showed any evidence of tumours. The effect of sex on response to NiS was tested by injecting 20 mg. into male and female Hooded and Wistar rats, while age differences were studied by the injection of 5 to 10 mg. into Hooded rats at ages of six hours, two days, nine days, three weeks and four months. In the age experiments, intramuscular injections into newborn and baby rats (up to nine days) proved almost impossible because of the small quantity of muscle available. However, an approximate dose, as indicated above, was injected in 0.05 cc. distilled water into the hind thigh area. The suspension was seen to spread beneath the skin over the entire ventral leg. Mortality was high in experiments involving young animals, so that many had to be repeated in order to obtain significant numbers of treated animals.

It was soon apparent that NiS was much more toxic than Ni_3S_2 and of less carcinogenic potency. After four months

only two tumours were visible and two others palpable as swellings in the injected muscle region. All were found in Hooded rats which had received 20 mg. of NiS at the age of four months. One tumour was excised for transplantation (which was unsuccessful), and showed some clumps of nickel throughout the tumour mass. The firm, white nodule was situated at the edge of the gastrocnemius muscle and appeared to be contained in a fibrous sheath which acted as the point of attachment to the gastrocnemius. The injected muscle still had a pocket of nickel which was grossly visible near one surface. Histological examination of both tumour and muscle revealed several features of interest which will be presented in another section.

Several subcutaneous swellings occurred in male Wistar rats which had received 20 mg. of NiS. These arose two weeks after injection and disappeared after two months. One which persisted developed into a firm mass approximately two cm. in diameter. Autopsy revealed that the gastrocnemius muscle had completely disappeared while a large, encapsulated and freely movable mass had become situated in that area. The interior of the capsule consisted of gritty particles of nickel mixed with necrotic, pale green material, the whole being surrounded by a fibromuscular covering.

Similar toxic reactions to the metal were seen in several young rats which had received 50 mg. of NiS. This high dose appeared to elicit overly toxic side-effects, both systemic and local. Approximately 30 per cent of the treated rats died within two weeks following injection, while two young rats developed a solid mass after two weeks, at the site of injection. These masses persisted for eight weeks, at which time the animals were killed and the swellings examined. The size at time of death was about two cm. in diameter. Autopsy revealed an outer capsule of fibrous tissue surrounding a necrotic mixture of nickel and inflammatory cells and fluid. Injection of the same dose into two hundred gram rats caused death to 40 per cent within three weeks after injection, although none developed similar swellings.

Serial Histological Study. In an attempt to study gradual effects of nickel sulphide on muscle, a serial study was undertaken in which 20 mg. of NiS was injected into forty-five male and female Hooded rats. Then, beginning four weeks after injection, rats were sacrificed at bi-weekly intervals and the treated muscles were examined for histological changes. Such changes began to be sporadically evident after eight weeks. Most of the muscles at four, six, eight, ten and twelve weeks

showed nothing unusual, although on gross observation it was noted that approximately 80 per cent of the rats had no observable nickel particles in the muscle area. One muscle at eight weeks had a small pocket of granuloma tissue surrounding the nickel particles, which bulged up from the external surface. The muscle showed abnormal histological detail, to be described later, which indicated possible malignant changes.

Transplantation

Several transplant lines were maintained in three different strains of rats. Those carried in Hooded and Wistar rats resulted from the present experiments while the Fischer transplants were obtained from Gilman, all having been induced by injection of Ni_3S_2 . In general the tumours exhibited markedly different gross characteristics among the three species. The Wistar and both Fischer transplants appeared solid and firm, with little evidence of necrosis. All Hooded lines, on the other hand, were firm only around the outside edge, with the inner area being soft and bloody. As well, local hemorrhage showed beneath the skin in that area. All transplants killed the animals in three to eight weeks and showed extreme necrosis at that time.

In a preliminary attempt to determine the minimum range of cells necessary for takes, one Fischer line was injected with dilutions of five million to sixteen thousand cells. Only one tumour had appeared in the five million cell range after fifty-five days, while all control tumours, which had received ten million cells, appeared after seven days. A similar experiment was performed on a Hooded line and it was found that 100 per cent tumours arose after thirty-nine days when four thousand cells were injected intramuscularly. The first tumour appeared on day twenty-eight as opposed to day sixteen for controls. Another experiment on this same tumour line involved centrifugation and separation of the tumour suspension before injection. Rats receiving the packed cells developed 100 per cent tumours after sixteen days while those receiving the supernatant, which was supposedly cell-free, as determined in a counting chamber, produced one tumour after fifty-seven days.

The various tumour lines maintained in the laboratory showed characteristic growth periods, as indicated by the time necessary to grow to a standard, measurable size of approximately one cm. Although some of the lines underwent changes on repeated transplanting, most growth rates were stabilized at distinctive rates, ranging from six to twenty-one days.

Histology

Histological examination of approximately one hundred sections stained with haematoxylin and eosin were carried out. Most of the sections examined were primary and transplanted tumours, with some attention being paid to various body organs. In addition to regular staining with haematoxylin and eosin (H and E), two special stains were used for identification of specific characteristics. One of these, Mallory's phospho-tungstic-acid haematoxylin, was used to stain approximately eighty sections for the presence of muscle elements. Since this solution, commonly labeled PTAH, stains striated fibrils blue, the stain was necessary in order to examine sections for cross-striations, not only on the muscle fibers, but also on neoplastic cells. An example of the desired result is shown in Fig. 1. A second stain which was used for means of identification was Perl's stain. The Prussian blue reaction resulting from this is a specific histochemical test for the presence of iron. In addition, other metals can be detected, such as nickel, which gives a greenish-white reaction. The latter stain was used on a few muscle and tumour sections and most injected organs in order to verify the continuing presence of nickel. Sections chosen for microphotography are stained with H and E, unless otherwise stated.

Several excellent references were used to aid in diagnosis of the pathological sections, including pathology texts by Willis (84), Robbins (72) and Adams, Denny-Brown and Pearson (1). In addition, the review on rhabdomyosarcomas by Stout (77) and articles by Bullock and Curtis (10) and Gilman (29) were used as sources of comparison for histological pictures of the various tumours. Recent examination of certain sections by Dr. W.L. Dunn served to clarify several cytological details.

The majority of tumours arising in Hooded rats injected with Ni_3S_2 showed a highly cellular and pleomorphic picture. Typical examples are seen in Fig. 2 and Fig. 3. Cells appear large and irregularly round or strap-shaped and elongated, and often exhibit many granules which stain deeply acidophilic with H and E. An atypical racquet cell is seen in Fig. 2. Faint suggestions of cross-striations are occasionally seen, but are probably due to arrangement of granules in a linear fashion. Among the more prevalent cell types are large cells with a foamy, vesiculated appearance. Fig. 4 illustrates some of these typical "spider-web" cells. Occasional giant cells resembling muscle fibers are noted and they often contain

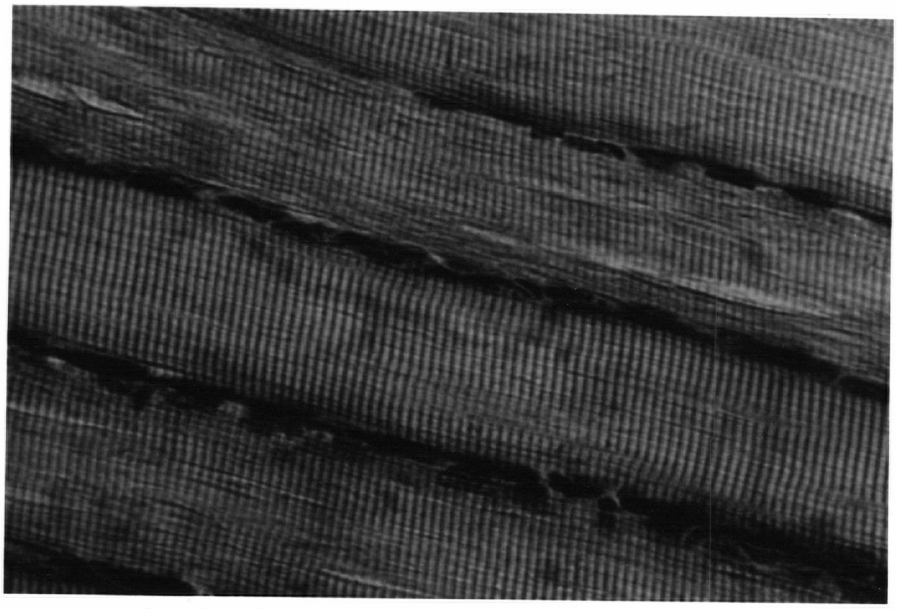

Figure 1

Normal Muscle Showing Striations. PTAH. x500.

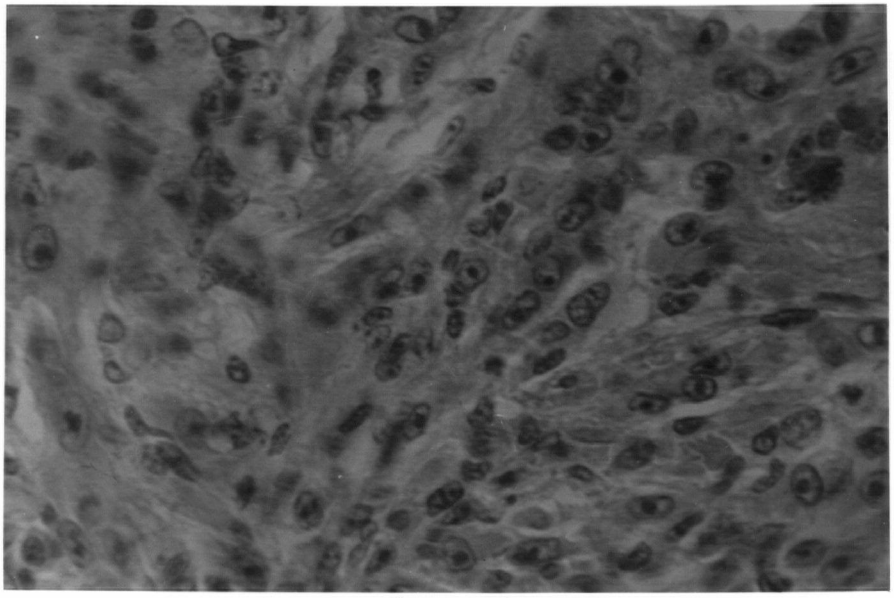

Figure 2

Ni_3S_2 Induced Primary Rhabdomyosarcoma. x500.

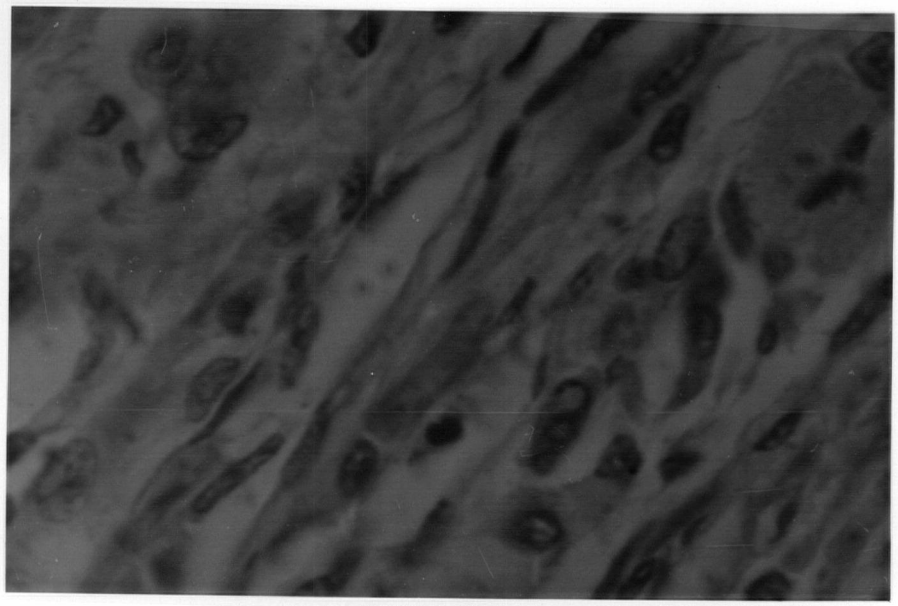

Figure 3

Ni_3S_2 Induced Primary Rhabdomyosarcoma with Strap Cells. x800.

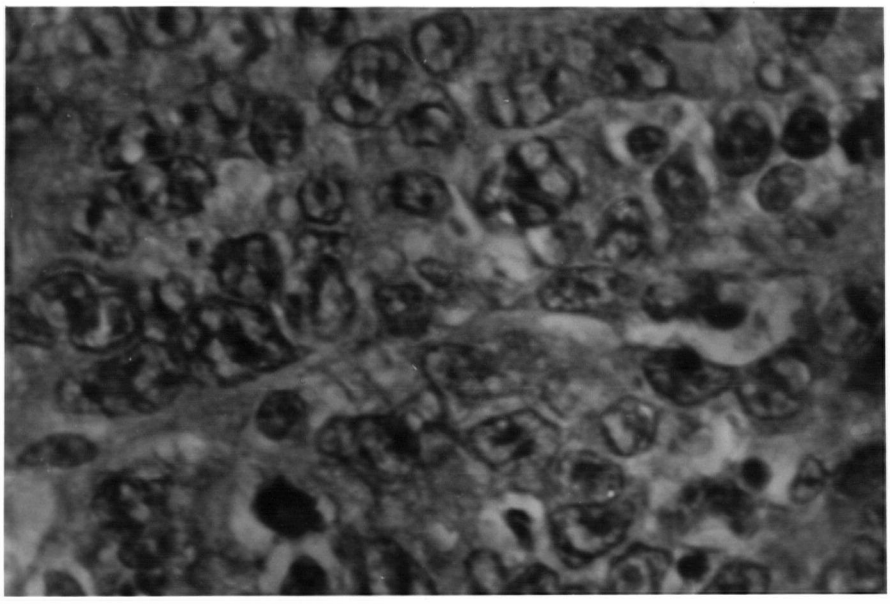

Figure 4

Ni_3S_2 Induced Rhabdomyosarcoma with Spider-web Cells. x800.

multiple nuclei and fibrillar stranding, as seen in Fig. 5. Another histological feature of the tumours is seen in Fig. 6, which indicates the presence of many mitotic figures. These generally exhibited phases of metaphase, anaphase or telophase and were found more often in primary tumours or during the first few generations.

One tumour which arose after eleven months appeared to have bundles of elongated fibers interspersed with round cells, as shown in Fig. 7. In view of its gross characteristics which were previously described and its histological appearance of locally invasive fibrosis among muscle cells, a tentative diagnosis of a benign tumour was made.

Only one tumour arose in Hooded rats in a body area other than muscle. A fifteen month old rat gave rise to a large, soft tumour in the lower right quadrant which appeared microscopically to have glandular formation. The presence of ducts filled with secretion suggested the formation of some kind of mammary tumour.

Tumours arising in Wistar rats consisted of two mammary neoplasms, one carcinoma of squamoid origin and one unidentified tumour. The multiple tumours which had developed in one rat showed glandular stroma, with ducts and little

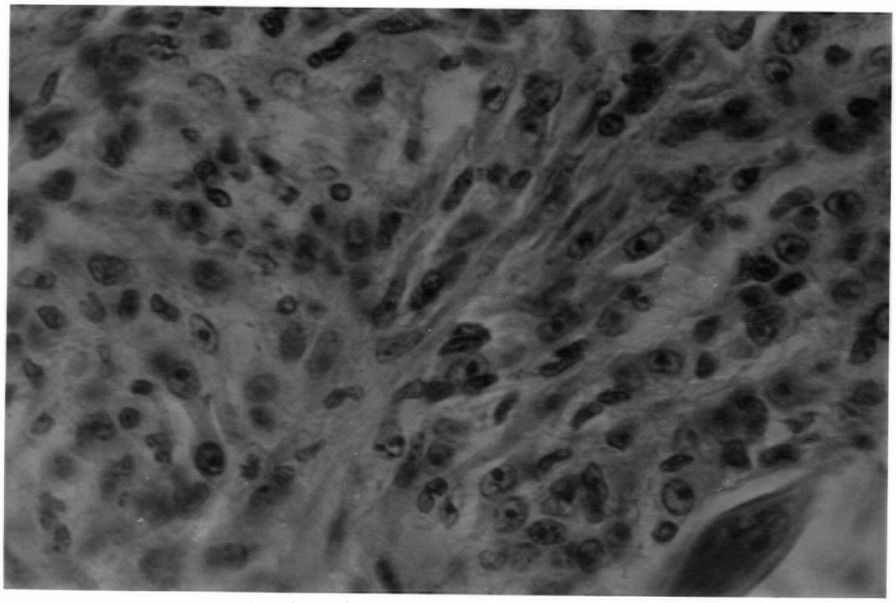

Figure 5

Pleomorphic Rhabdomyosarcoma with Giant Cell. x500.

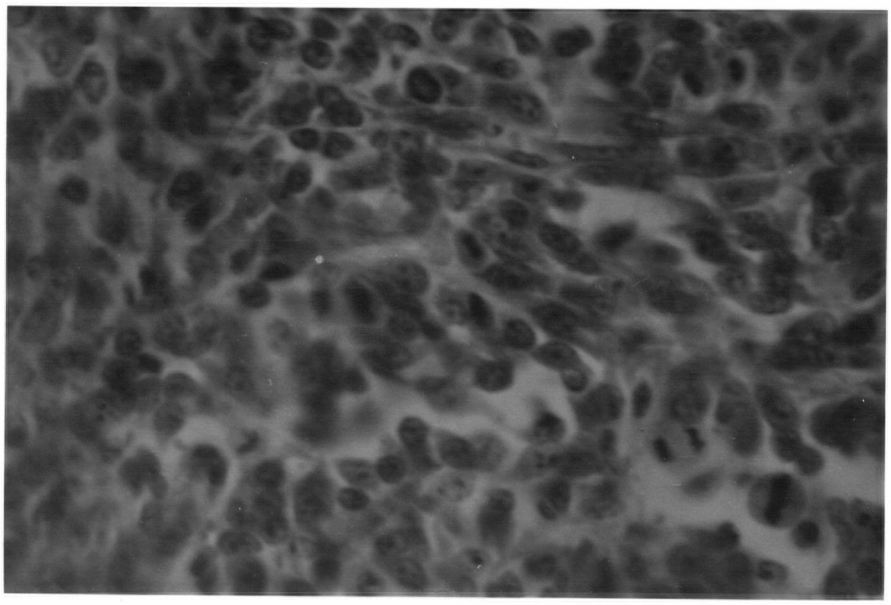

Figure 6

Ni_3S_2 Induced Rhabdomyosarcoma with Mitotic Figures. x500.

secretion, indicating mammary origin, while the tumour arising in the neck was not identifiable. The 7-N tumour arising in the leg region had a histologic appearance dissimilar to rhabdomyosarcomas. Cells were larger and rounder with no spindle shapes evident. Nuclei were paler and vesicular and cells seemed to be arranged in broad sheets. Such characteristics indicated a tumour of squamoid origin such as a squamous cell carcinoma.

The sectioned tumour and muscle resulting from injection of NiS showed several interesting features, as seen in Fig. 8. The tumour appears more fibrillar than the other cellular forms noted previously, and a large amount of the nickel is still scattered throughout the stroma. No giant cells and very few mitoses were seen. It is possible that the tumour may have been a benign rhabdomyoma, similar to Fig. 7. The disruption of the muscle fibers which had been injected with NiS is shown in Fig. 9 and indicates the damaging effect of the metal. Individual fibers show complete disarrangement and are surrounded with many fibroblasts and lymphocytes as well as nuclei from degenerated muscle fibers. Some multiple nuclei in tandem are seen in an elongated fiber, suggesting myoneural tube formation.

Certain histological results of the serial study gave tenuous indication that a neoplastic process might have occurred

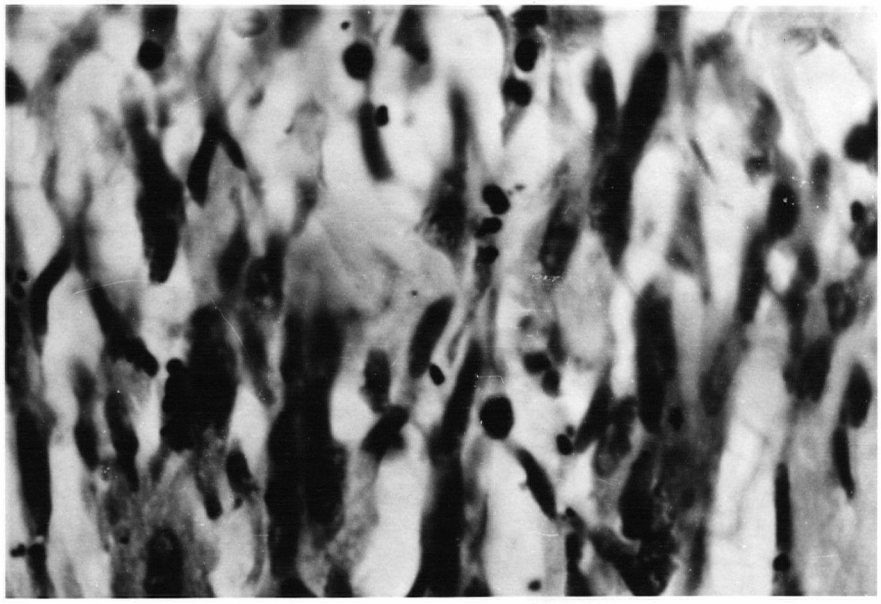

Figure 7

Benign Fibrous Tumour Induced by Ni_3S_2. x125.

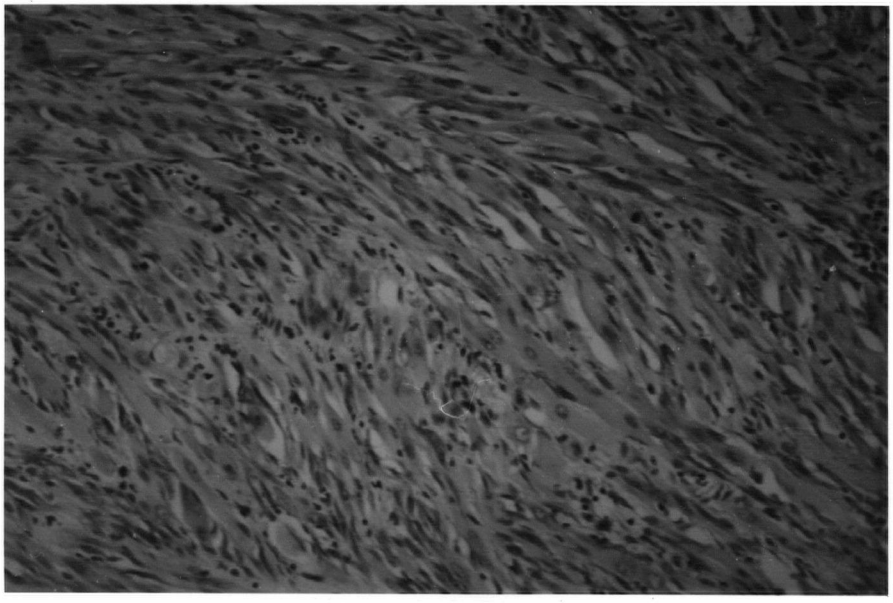

Figure 8

Fibrous Rhabdomyoma Induced by NiS. x500.

after eight weeks. Some muscles examined at four weeks had clumps of nickel in the muscle mass but the tissue itself did not appear to be affected beyond a slight increase in lymphocytes. The lack of nickel noted grossly in most muscles examined at four to twelve weeks was substantiated by histologic study of the treated areas.

Fig. 10 shows the muscle which had nickel present after eight weeks and it is seen that there is fiber disarrangement somewhat similar to that described in Fig. 9. The solid clump of nickel was surrounded by a wall of lymphocytes and did not appear to be interspersed throughout the whole muscle, although histochemical staining did show some particles in fibers some distance removed from the solid clump. Fig. 10 shows an area near the nickel focus in which there seems to be a mingling of cell types. Fragments of striated fibers are mixed with individual muscle nuclei and small cells, indicating atrophy of fibers. Other isolated fibers, not pictured, appeared to be active rather than atrophic, giving the appearance of giant cells in formation. Nuclei were large and vacuolated with dark nucleoli, and cytoplasm was darkly stained with eosin, indicating protein activity. Significance of these findings is discussed later.

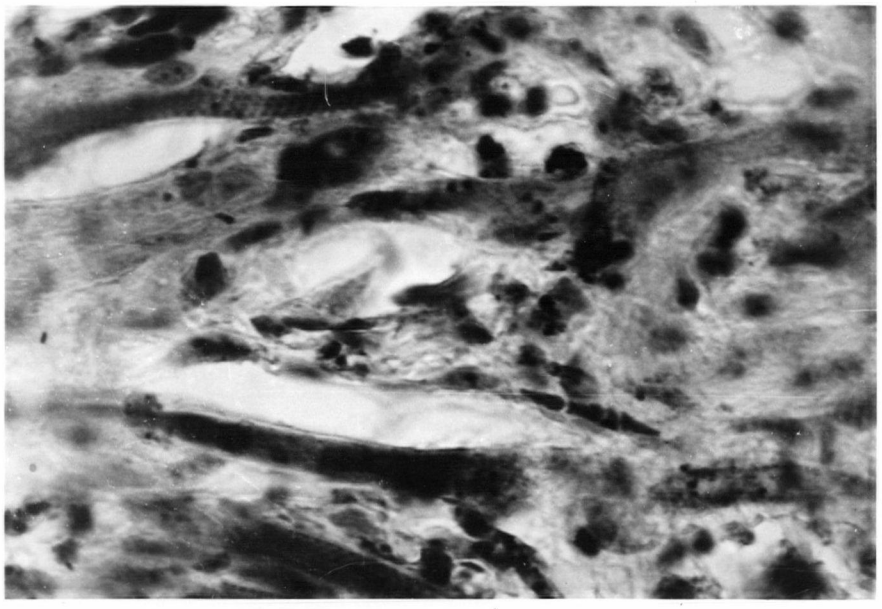

Figure 9

Disrupted Muscle, after Injection with NiS. PTAH. x500.

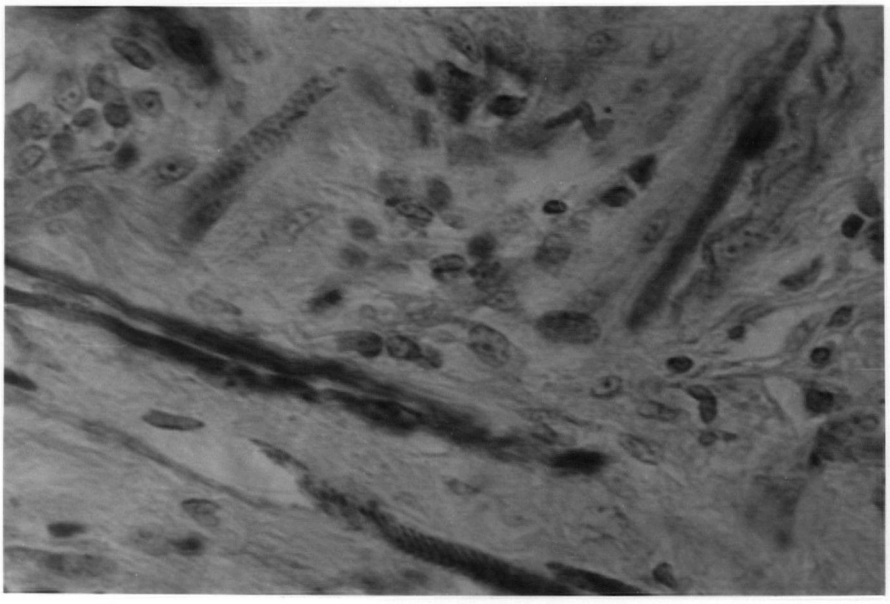

Figure 10

Muscle, Eight Weeks after Injection of NiS. PTAH. x500.

Injections into various organs resulted in several unusual histological findings. Examination of the tumour arising in those rats which had received an intra-hepatic injection indicated that it, too, was a rhabdomyosarcoma. The pattern was generally similar to the leg muscle tumours although somewhat less anaplastic. The suggestion of peritoneal origin of the tumour was confirmed by a section of the tissue adhered to the abdominal wall. As seen in Fig. 11, the neoplastic tissue is continuous with the striated layer of the abdominal wall. Fig. 12 shows the attachment to the gastrointestinal tract and also demonstrates some of the pleomorphic cell types.

A comparative examination of testes from rats which had received different treatments indicated the relative potency of nickel sulphide. Although the rats with intratesticular injections showed no tumour formation, intense damage to testicular tissue was noted. As seen in Fig. 13, the seminiferous tubules are almost completely destroyed with only an occasional row of spermatogonia remaining. The cell-free tubules have patches of material in them which exhibit an eosinophilic reaction with periodic acid Schiff reagent. The interstitial spaces show much phagocytic activity, with many particles engulfed in histiocytes, as seen in Fig. 14.

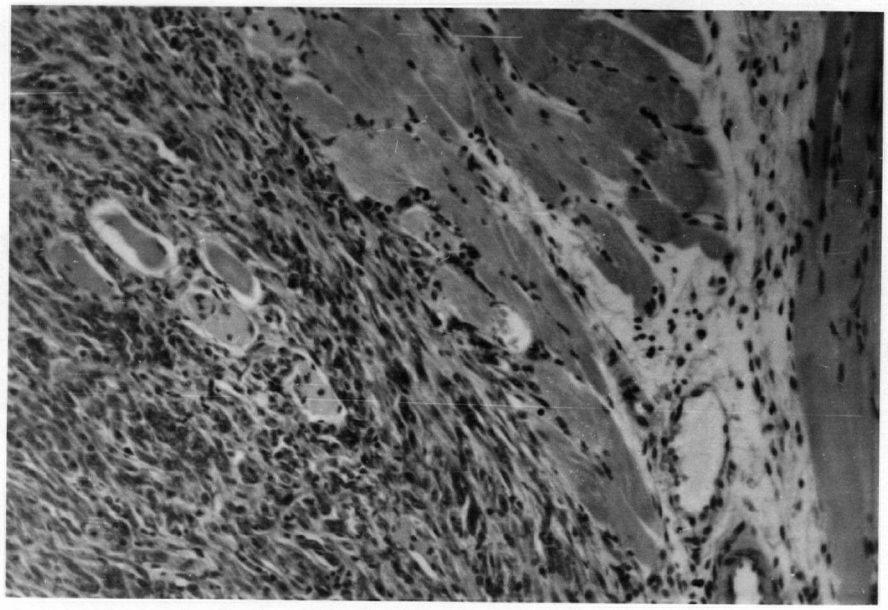

Figure 11

Attachment of Tumour to Peritoneal Wall after Intra-hepatic Injection. x125.

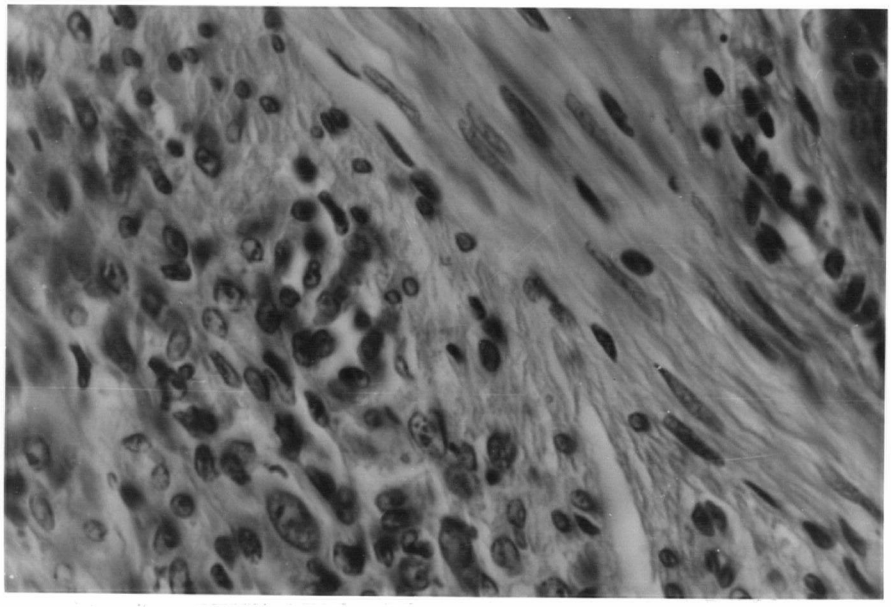

Figure 12

Attachment of Above Tumour to Gastrointestinal Tract. x500.

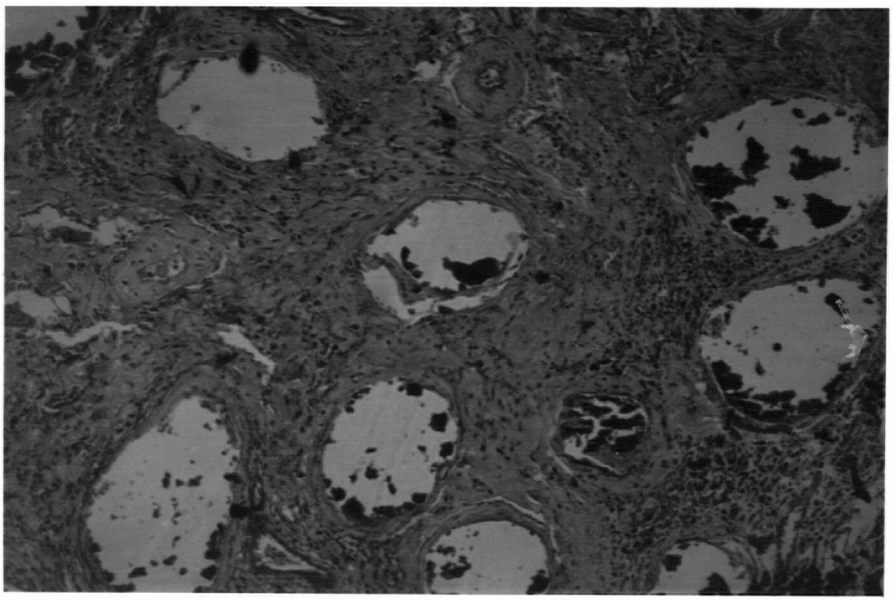

Figure 13

Testicular Tissue Six Months after Injection of Ni_3S_2. PAS. x50.

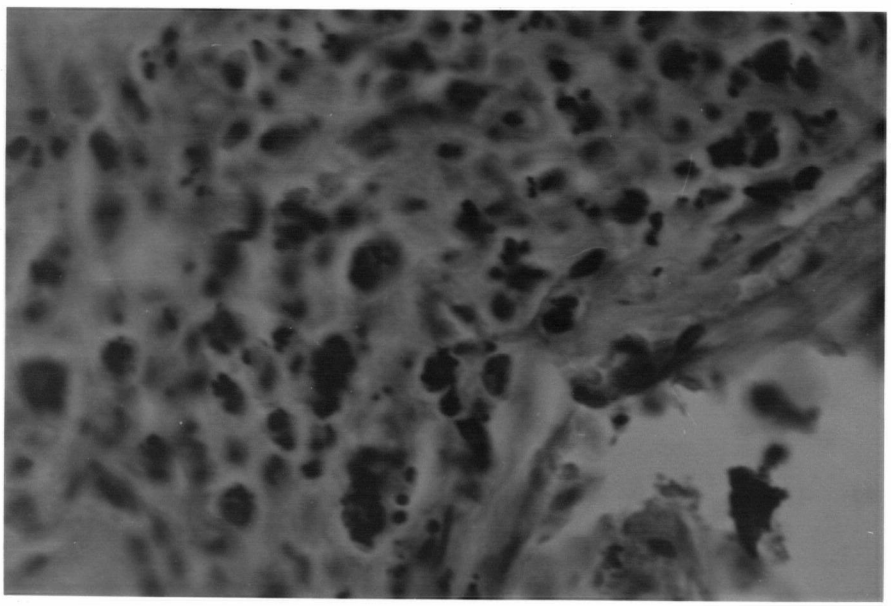

Figure 14

Macrophages and Nickel Particles in Interstitial Spaces of Above Tissue. x500.

Nickel was demonstrated histochemically to be not only in the particulate form but actually dissolved throughout the macrophages. A second injected testis showed similar tubular degeneration but, in addition, there was an area of solid collagen and fibroblasts with no tubules present, indicating previous complete destruction supplanted by fibrous growth. Around the edge of the fibrous mass was a ring of dystrophic or metastatic calcification.

The testis of the prostate-injected rat showed slight tubular atrophy, but of such a degree as to implicate age of the animal rather than effect of the nickel. The only probable reaction to the metal in the prostate was the presence of some interstitial cell edema, such that some Leydig cells were separated by a greater amount of stroma. The testis of a Hooded rat injected intramuscularly with NiS showed no abnormalities at all.

Examination of the splenic nodule revealed several intriguing details. First of all, a large number of mitotic phases were present, including abnormal chromatin bridge figures. Also there were some giant cells with multiple vesicles which might have been either giant fibroblasts or abnormal pre-neoplastic cells. The main bulk of the nodule

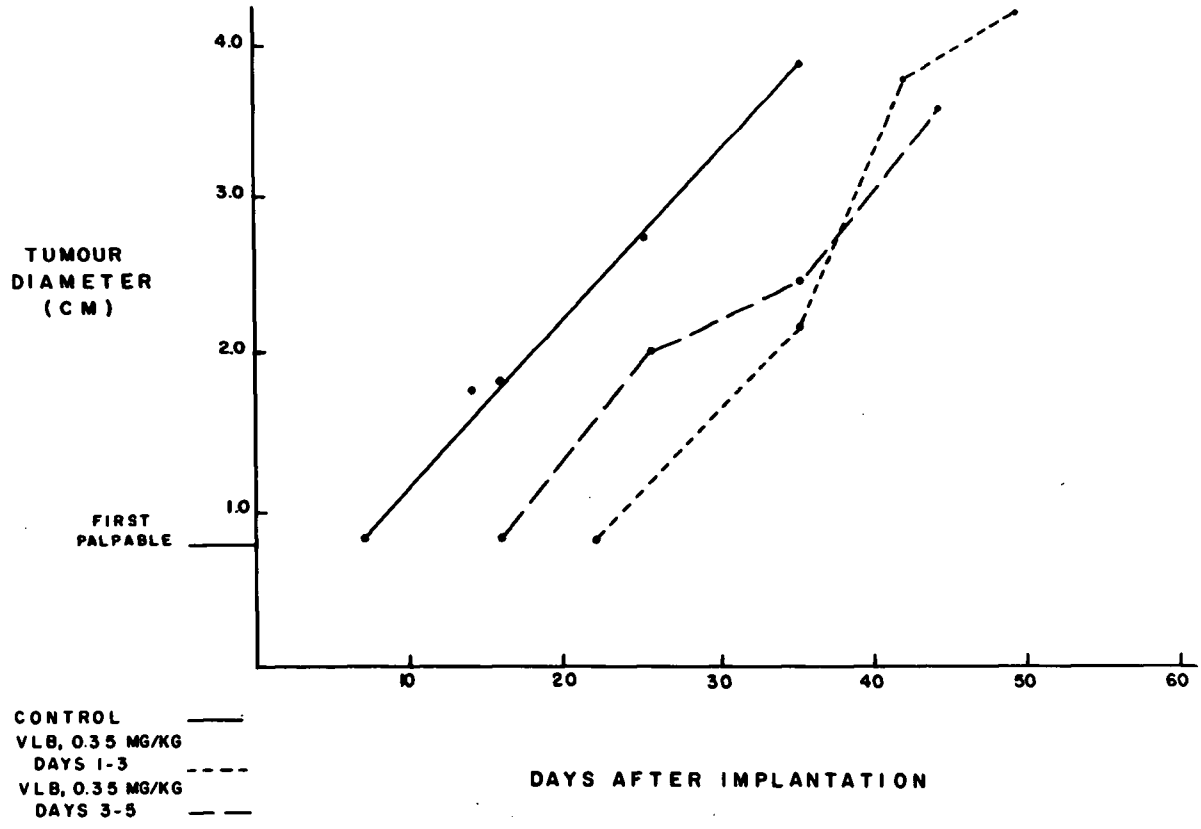

Figure 15

Inhibition of growth rate of 2-N-5 following administration of Vincaleukoblastine during first week after implantation.

consisted of a loose meshwork of fibroblasts with some foci of calcification.

The injected uterus showed metastatic calcification surrounding nickel particles in various areas. Although the myometrium seemed to have an excessive amount of collagen, the cellular appearance was not unusual. Injected ovaries had no observable nickel and appeared normal, with functioning follicles.

Therapeutic Studies

Alkaloid Treatment. Vinblastine was tested at random on several transplant lines. Dose regimens were varied, as were times of administration. Tumour diameters were measured throughout the injection period and at four to six weeks after implantation. Beyond this time period, factors such as necrosis tended to confuse results. Table II lists summarized results.

Response varied considerably in the different tumour lines, with the best effects being noted in the 2-N line. The tumours occurring in treated rats grew during the injection period but at a slower rate than in control animals. An example of this is shown in Fig. 15. It is of interest to note that the most effective suppression of takes was observed in those rats bearing the 2-N tumour which received three consecutive

TABLE II

RESPONSE OF RHABDOMYOSARCOMAS TO VINBLASTINE

Tumour line and generation	Dose	Tumour diameter (mean ± S.D., cm.)	Number of tumours
2-N-5	Controls	2.7 ± .2	4/4
	0.35 mg/kg days 1-3	2.1 ± .1	2/4
	days 3-5	1.8 ± .3	2/4
	days 6-9	3.2 ± .7	4/4
	days 14-24	1.9 ± .1	3/4
5-N-18	Controls	2.4 ± .1	4/4
	0.1 mg/kg days 1-10	2.6 ± .5	4/4
10-N-2	Controls	2.2 ± .3	4/4
	0.3 mg/kg days 1-10	(all rats dead by day 15)	
12-N-2	Controls	2.2 ± .4	5/5
	0.1 mg/kg days 1-10	2.2 ± .2	5/5

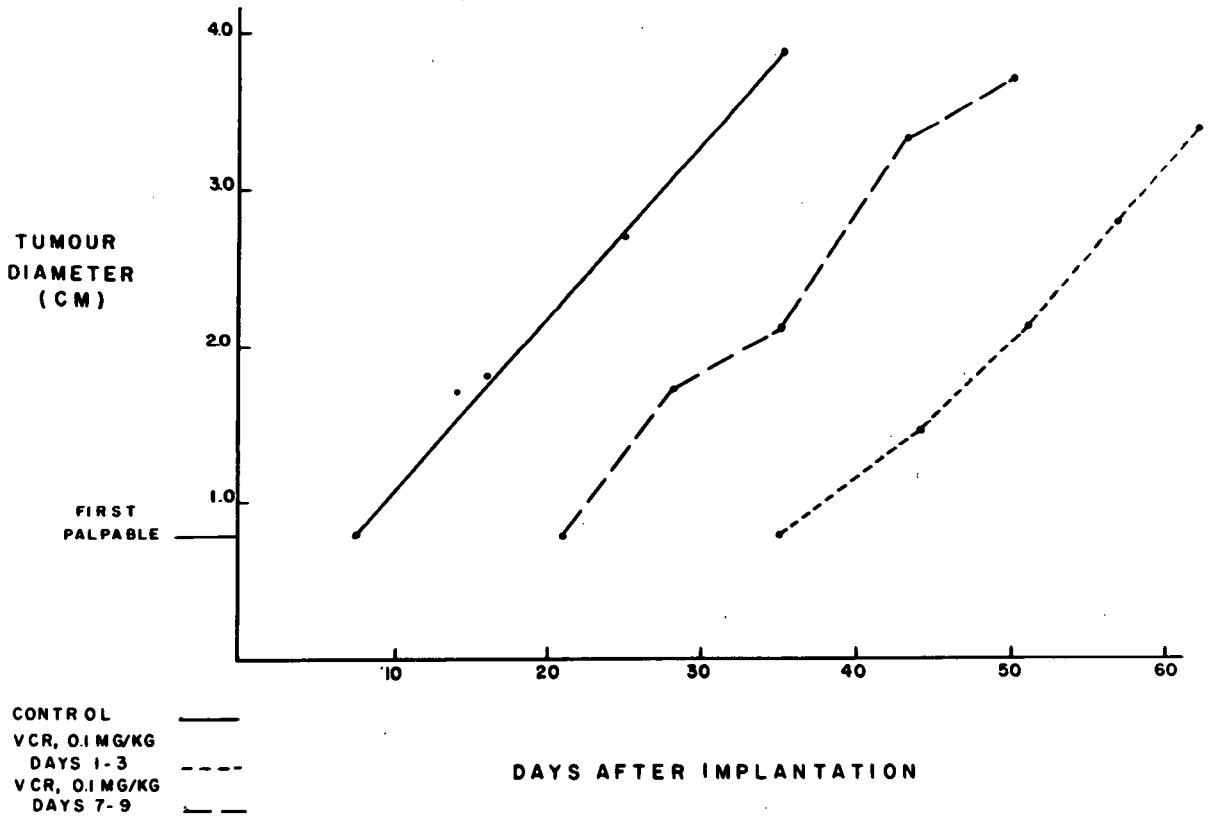

Figure 16

Increase in latent period for 2-N-5 following administration of Vincristine.

injections of Vinblastine beginning one or three days after transplantation. Those animals in which tumours did not occur at four weeks time had not developed tumours by the termination date of seven weeks. The significance of these results will be discussed later.

Vincristine was administered to the same tumour lines in varying dose regiments. Results are summarized in Table III. An increased latent period was demonstrated by the injection of Vincristine to 2-N-5 during days one to three. The effect was made more emphatic by the fact that the first tumour arising from five treated rats did not become palpable until forty-three days after treatment had been initiated. The remaining treated rats showed small tumours after fifty-one days, thus the test to control ratio was 1:6 or 0.17. Effective response to the alkaloid decreased as treatment was delayed up to seven days, illustrated graphically in Fig. 16. It is also seen that growth appeared to be somewhat inhibited during the first three weeks with a proliferative spurt being shown after that time.

Hormone Administration. All rats bearing transplantable rhabdomyosarcomas were treated with cortisol, cortisone or prednisolone, in regimens chosen at random in an attempt to find tumour lines sensitive to adrenal corticoids. All injections were begun seven days after implantation, when most

TABLE III

RESPONSE OF RHABDOMYOSARCOMAS TO VINCRISTINE

Tumour line and generation	Dose	Tumour diameter (mean ± S.D., cm.)	Number of tumours
2-N-5	Controls	2.7 ± .2	4/4
	0.1 mg/kg days 1-3	1.7	1/5
	0.1 mg/kg days 7-9	1.7 ± .6	4/4
	0.1 mg/kg days 14-24	2.2 ± .5	4/4
5-N-18	Controls	2.4 ± .1	4/4
	0.1 mg/kg days 1-10	2.4 ± .5	4/4
10-N-2	Controls	3.2 ± .3	3/3
	0.1 mg/kg days 1-10	2.9 ± .2	4/4
12-N-2	Controls	2.2 ± .4	4/4
	0.1 mg/kg days 1-10	2.0 ± .1	4/4

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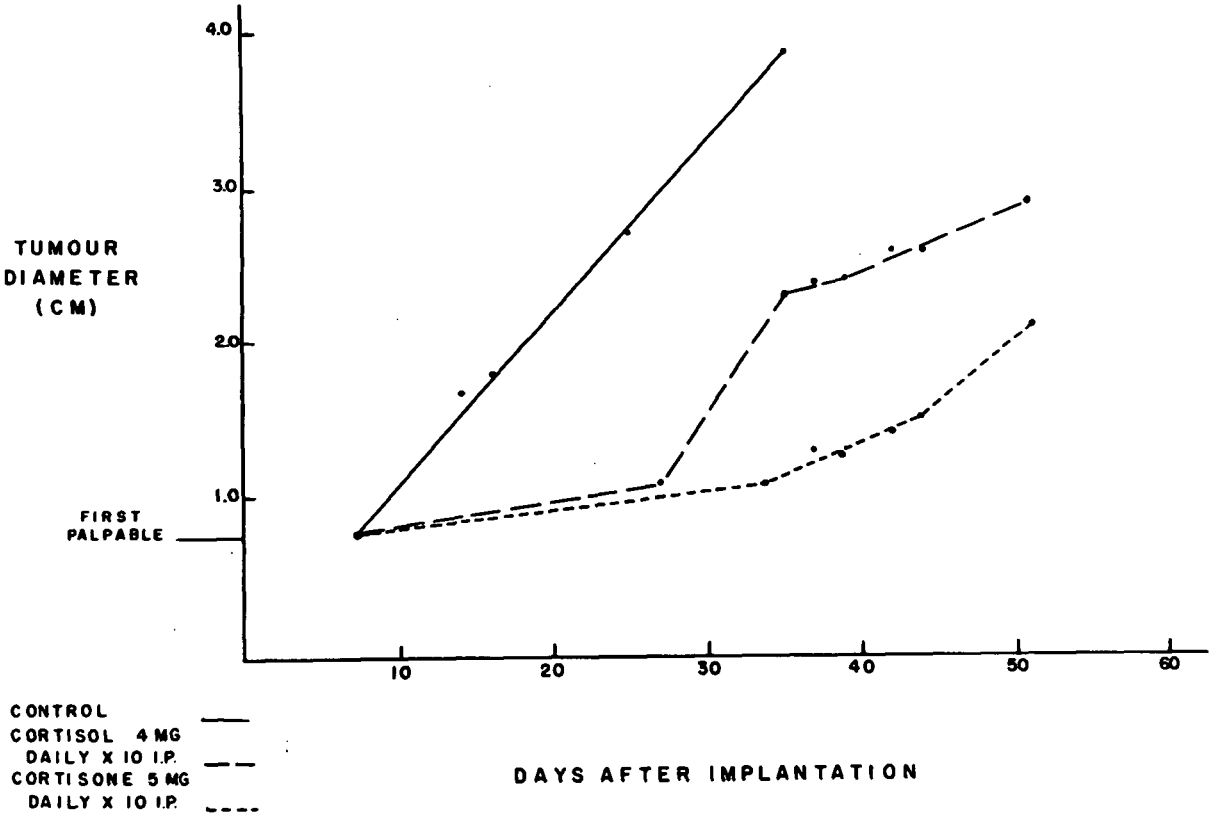

Figure 17

Suppression of growth of 2-N-5 during the intraperitoneal injection of cortisol or cortisone. Injection period for steroids, days seven to seventeen.

tumours were just measurable. Several dose regimens and results are given in Table IV.

Positive response, indicated by varying degrees of inhibition of growth, was obtained in four out of seven tumour lines tested in both Hooded and Fischer rats. Intraperitoneal injections of 40 mg. of cortisol and 50 mg. of cortisone resulted in complete suppression of tumour growth in 2-N-5 during the injection period, as seen in Fig. 17, although similar cortisol treatment of the same tumour in the twenty-third generation resulted in reversal of effect. The appearance of tumours in treated rats in the latter experiment was actually slightly larger than those in control rats. Subcutaneous administration of the corticoids was adopted in the majority of experiments, and some inhibition of growth was achieved in all experiments using 2-N, 8-N and 12-N. Generally the inhibition was residual in that tumour growth was not completely inhibited but was slowed during the injection period tothe extent that treated rats had tumours which were one-third the weight of control tumours. Weight gain was generally stopped or reversed during injections, although rats gained weight after treatment was discontinued.

Testosterone was administered in a standard dose of 5 mg. injected subcutaneously for ten days to 2-N, 6-N, 7-N,

TABLE IV

EFFECTS OF STEROIDS ON RHABDOMYOSARCOMAS AND BODY WEIGHT

Tumour	Number of rats	Steroid	Dose and route	% change body weight	Tumour size grams	T/C
2-N-5	5	Controls	------	+ 39.9	36.30	
	5	Cortisol	40 mg. I.P.	+ 13.6	3.61	.09
	5	Cortisone	50 mg. I.P.	+ 18.9	2.83	.08
	5	Cortisone	50 mg. S.C.	- 13.6	15.85	.44
2-N-8	5	Controls	------	+ 3.7	59.05	
	5	Cortisol	40 mg. S.C.	- 9.8	14.16	.24
	5	Cortisone	50 mg. S.C.	- 17.8	18.59	.31
2-N-16	5	Controls	------	+ 12.2	18.57	
	5	Cortisol	20 mg. S.C.	- 7.4	5.44	.29
	5	Cortisol + testosterone	20 mg. S.C. 50 mg.	+12.2	5.29	.28
2-N-23	5	Controls	------	+ 12.9	3.25	
	5	Cortisol	60 mg. I.P.	+ 5.1	7.33	2.26
4-N-16	5	Controls	------	+ 32.0	169.87	
	5	Prednisolone	20 mg. S.C.	-13.2	28.51	.17
8-N-10	5	Controls	------	+ 13.0	58.81	
	5	Cortisol	40 mg. S.C.	- 9.0	17.24	.30
12-N-2	5	Controls	------	+ 37.6	21.90	
	5	Cortisol	40 mg. S.C.	+ 4.0	5.53	.25

8-N, 10-N and 12-N. Time of initiation was an important variable, with most animals receiving the first injection on days one or seven after implantation, as seen in Table V. Two out of four groups injected from days one to ten showed accelerated growth of tumours, while no groups receiving injections beginning on days four or seven showed any significant difference in size between test and control tumours. One exceptional result was noted in 2-N-19, when early treatment with testosterone resulted in great inhibition of growth, a result which was markedly different from that obtained when the same treatment was given to the same tumour line three generations earlier.

2-N-16 received combined treatment of 2 mg. of cortisol and 5 mg. of testosterone, both given ten times subcutaneously beginning day seven. This resulted in some inhibition of tumour size, similar to that seen with cortisol alone, but with an increase in weight equal to that seen in control animals (see Table IV).

TABLE V

EFFECT OF TESTOSTERONE ON RHABDOMYOSARCOMAS

Tumour line and generation	Number of animals	Treatment initiated (days after transplant)	Response T/C
2-N-9	5	1	.62
2-N-16	5	4	1.00
2-N-19	5	1	.08
6-N-11	5	1	1.64
6-N-12	5	7	1.15
7-N-11	5	1	1.74
8-N-10	5	7	.83
10-N-2	5	1	.76
10-N-3	5	7	.61
12-N-2	5	7	.67

DISCUSSION

There are certain acknowledged problems attendant in using animals for tumour studies. Tumour induction particularly can be inhibited by environmental factors such as inadequate diet, general health, infections and so on. Other variables occur in selection of the species, strain and sex of the experimental animal chosen, and the age at the start of the experiment (8). Even if these factors are carefully regulated, results may still vary considerably. Hieger states the discouraging fact that tumour induction assays are often reproducible only by a factor of two or five (42). That is, one may find 10 per cent tumours the first time, and only 5 or 2 per cent the next time, in spite of identical technique.

When working with a compound of unknown activity, relatively empirical choice of dose, route and rate of administration is necessary since its general toxicity to the animal is not known. It is usual to assay a compound for toxicity before beginning the actual experiments. In the case of Ni_3S_2 the original assay tests were performed by Gilman, who found that injection of 30 mg. into a muscle caused some local effects probably due to trauma (26). It was possible to use 20 mg. per muscle without adverse effects, although bilateral injections of this dose sometimes resulted in general toxicity.

A suggested reason for this was the presence of nickel sulphate, a salt which was easily removed by washing the injection mixture.

The induction of tumours by intramuscular injection of Ni_3S_2 proved to be fairly reliable. The short latent period of ninety-two days exhibited in those rats which had received 20 mg. suggested that this compound had strong carcinogenic activity in Hooded rats. That the latent periods obtained were shorter than those recorded by Gilman after injection of the same dose into Wistar rats might be due to the fact that Hooded rats are highly inbred whereas Wistars are not. It has been found so far that none of the inbred strains is completely resistant to a carcinogenic stimulus, if the species in general is known to be susceptible to such a stimulus (5). Since genetic factors may control the degree of susceptibility, it is feasible that a carcinogen may elicit tumours more readily in the highly inbred strains than in those which are not inbred.

There seemed to be some correlation between amount of nickel sulphide injected and percentage tumour response in relation to time. The most rapid induction was achieved in four months when 100 per cent tumours occurred after injection of 20 mg. From Table I, it was seen that tumour appearance

was 100 per cent with 10 mg. as well, but with 2.5 mg. and 0.5 mg. it decreased. It may have been that there was not enough nickel available after a certain length of time. Gilman has stated that the compound is slowly soluble and diffuses out gradually over a long period of time (27). The injection of higher doses perhaps meant that greater amounts of nickel were present to attack a proportionately greater number of cells, thereby setting up different and larger foci of altered cells throughout the muscle, whereas a small dose might have had only enough nickel to set up one small focus. In the rats given 2.5 mg. and 0.5 mg., the rate of increase in per cent response appeared to slow down after eight months, possibly because the absolute amount of nickel present at that time was critical.

This hypothesis might explain the fact that the average latent period was not significantly different in those rats injected with 0.5 to 10 mg. Although the expected delay in tumour appearance was seen between 10 and 20 mg., among the lower doses only twenty days separated the average latent periods. This again indicated that the absolute amount of nickel was the deciding factor, once the concentration was below a critical level. Above this theoretical amount, an

increase in number of nickel particles might result in yet faster induction of tumours. Whether or not the two-stage mechanism of carcinogenesis (25) could be applied to this situation is strictly a matter of speculation. It would involve a critical amount of nickel being needed to convert normal cells to subthreshold neoplastic cells (initiating stimulus), with a further and larger amount of nickel neede to stimulate such cells to proliferate into a visible tumour (promoting stimulus).

As mentioned in the introduction, tumours of striated muscle origin have been extremely rare in experimental animals. The identification of such tumours induced by Ni_3S_2 as being rhabdomyosarcomas was made on the basis of several observations. First, the site of origin was invariably in the leg muscle region, with the tumour being situated in the area of the gastrocnemius muscle. Second, the fact that they were fast-growing, once palpable, and exhibited rapid lethality indicated their malignant or sarcomatous nature. The third and most positive means of identification was by histological techniques. Reference has been made to detailed descriptions of rhabdomyosarcomas by Stout (77) and Adams, Denny-Brown and Pearson (1). They have outlined certain specific types of rhabdomyoblast

cells which characterize these tumours: (1) large round cells with one to several nuclei; (2) strap cells, with two or more nuclei; (3) racquet cells with a single nucleus at one end and tapering body; (4) giant "spider-web" cells with a single nucleus and peripheral vacuoles separated by delicate cytoplasmic threads. Stout noted that cross-striations and longitudinally arranged myofibrils or some vague suggestion of their formation were sometimes seen in strap cells. This latter characteristic is considered to be absolute proof of a rhabdomyosarcoma, according to authoritative pathology texts (72, 84). However, many descriptions of rhabdomyosarcomas have mentioned that striations are notoriously hard, if not impossible, to demonstrate in some tumours.

Diligent searching of primary and transplanted tumour sections stained specifically for fibrils failed to reveal such striations. Gilman had stated that he found them only in the original tumours and not in any transplant generations (26). The fact that certain of the characteristic cellular elements were seen in all induced tumours gave histological support to the gross observation of rhabdomyosarcomas resulting from injection of nickel sulphide.

The observation that the Wistar rats, in contrast to Hooded, showed almost no response to a similar dose of Ni_3S_2 was rather curious, especially in view of the fact that rhabdomyosarcomas have been induced in Wistars by Gilman. There is some question as to the origin of the Wistar rats supplied at U.B.C. and the lack of tumours in these Wistars, as opposed to those used by Gilman which had been obtained from Woodlyn Farms at Guelph, might be explained in this way. The fact that no nickel particles were present in the injected muscle region suggested that the metallic compound may have been handled differently in this strain of rats. The original swellings which appeared between eight and twelve weeks might have been a local reaction of the tissues to the foreign irritant, resulting in its complete removal. It should be noted that the single Wistar rat which developed a benign tumour in the leg region had not shown a swelling in the third month, nor had the rat which developed the lump in the neck area. The nickel in these animals may have been distributed, at least theoretically, throughout the body, thus being able to elicit a systemic action.

It is possible that in most Wistar rats the nickel was dissolved and phagocytosed, then absorbed into the blood stream to be disposed of by excretion, although there seems

to be no logical reason why this should happen in only one strain of rats. Gilman has made preliminary studies on the excretion of nickel in urine after implantation of a disc of Ni_3S_2 which releases an amount of nickel equivalent to the injection of 10 mg., and has found that 3.5 per cent of the nickel was excreted after three months. 5 and 13 mg. of nickel combined with E.D.T.A. in pellet form resulted in the excretion of 38.6 and 27.3 per cent of the nickel, respectively. Although the results are equivocal, they suggest that nickel can be fairly easily excreted through the kidneys, even when in chelated form.

The injection of Ni_3S_2 into various body organs served to emphasize the destructive potentialities of this compound, as well as indicating a possible carcinogenic specificity for striated muscle. The latter point was suggested in the results of the intrahepatic injections. The extensive tumours which developed were almost certainly not of hepatic origin since the root of the tumours appeared grossly and histologically to be in the muscle layers of the peritoneal wall. It is probable that the suspension of nickel particles was extruded from the liver by leakage and somehow became adsorbed onto the muscle wall. The fact that it could adhere to the immediate

overlying portion of the peritoneum rather than being dispersed throughout the abdominal cavity is concomitant with Gilman's hypothesis that nickel has a specificity for striated muscle. The tumours did appear to be rhabdomyosarcomas and demonstrated extreme malignancy.

Although injections into other organs did not result in tumours, damage to most tissues was extensive. Testicular tissue seemed to be particularly sensitive to the presence of Ni_3S_2 located either directly in the area or in the prostatic tissue. The extreme degeneration of the seminiferous tubules, accompanied by much macrophage activity, which resulted from direct intratesticular injection was understandable as a drastic response to an extremely irritating local foreign substance. The partial effect on the testes in rats receiving intraprostatic injections could possibly have been due to some nickel circulating through the organ from the prostatic foci. However, the mild interstitial edema observed can be elicited by many irritant substances and was probably not a specific reaction to nickel.

The effect of direct contact of nickel with the testes is of interest in view of results that have been obtained with the use of other metals, such as cadmium. Kar and Das

have recorded in detail the testicular changes in rats following treatment with cadmium chloride (52). They observed that between two and seven days after subcutaneous injection the seminiferous tubules shrank and all cellular elements disappeared, with the interior of most tubules containing only dead cell debris. The interstitial cells disappeared after thirty days and were replaced by fibroblasts. No mention was made of phagocytic activity. The above picture of testicular damage is not unlike that obtained in the present experiments, particularly with reference to the presence of numerous fibroblasts and irregular patches of intra-tubular debris.

The presence of some abnormal cell forms in nickel-injected testes gives tentative suggestion that such tissue might have become neoplastic after a greater length of time, since testicular tumours have been induced by other metals. As reported by Rivière, zinc salts are commonly known to produce tumours of the testes in birds, provided that a gonadotrophic factor is present (71). Similar tumours have been induced by parenteral injection of zinc chloride in rodents. Rivière found that a 1 to 4 mg. suspension of this salt resulted in eleven tumours, most of them interstitial cell neoplasms, among one hundred and twenty-five rats after

two years. It is not improbable, then, to suggest that tumours might also have been induced by Ni_3S_2 after a longer exposure period. The fact that none were seen in the present experiments could also be due to the toxicity of the metal, since the dose of 5 to 10 mg. was obviously too damaging to most of the cells.

The tumour induction studies with NiS were intended to be an extension of the experiments using Ni_3S_2, in order to elucidate further variables affecting metal carcinogenesis. It was soon apparent that the different composition of this second form of nickel sulphide might mean that the compound would have different properties. NiS was obviously more toxic than the first compound, causing both systemic and local reactions. The latter response, manifested as local swelling, indicated the effectiveness of the host's defense against a highly irritant substance. Although the original muscle in these areas was sometimes completely atrophied, the nickel itself was contained within an isolating capsule. The presence of muscle fibers interspersed among connective tissue fibers suggested that the pocket of injected particles had probably digested most of the muscle tissue surrounding it.

The appearance of two definite measurable tumours among twenty Hooded rats injected with 20 mg. of NiS gave a

tumour response of only 10 per cent after four months, compared with 100 per cent using Ni_3S_2, suggesting that although NiS is capable of being carcinogenic, its potency is apparently less than that of Ni_3S_2. An unknown effect of age may have had something to do with the occurrence of tumours with NiS, since any tumours arising to date have been in rats which were injected as adults.

Although only one NiS-induced tumour has been examined histologically, it is interesting to speculate that this compound may have a slower and less potent effect. The suggestion that it has induced a benign rhabdomyoma, rather than a malignant rhabdomyosarcoma, offers potential experimental studies. Close surveillance of developing tumours by means of periodic biopsies might indicate whether the tumours could progress from benign to malignant states or whether they would originate in one state or the other and remain that way. The possibility that NiS has different carcinogenic abilities will be developed in future investigations.

In attempting to follow the action of NiS injected intramuscularly, difficulties were encountered owing to the unpredictable fate of the metal. Unlike Ni_3S_2, NiS did not remain in the original muscle area for any length of time, as noted in the results. In those muscles in which nickel was

still present six weeks after injection, the accumulation of lymphocytes was undoubtedly elicited by the chronic presence of a foreign irritant. The results obtained from one muscle which had nickel present at eight weeks indicated that the compound might be exerting carcinogenic capabilities at this time. Although there was no gross evidence of tumour formation, the presence of cells distinctively different from normal muscle fibers and similar to those seen in established rhabdomyosarcomas suggested that the muscle was undergoing pre-malignant changes. Gilman has found a similar picture of an increase in the number of subsarcolemmal nuclei with clusters of nuclei in degenerating giant muscle cells (30). Hueper had observed in his experiments involving intrafemoral injections of metallic nickel that around the nickel deposits there occurred proliferation of hyperchromatic, irregular cells, which he felt represented early sarcomatous foci (46).

Heath has performed an extensive study on the carcinogenic activity of cobalt in muscle, in which changes were observed and recorded from one day to twenty weeks after injection (39). Briefly, these consisted of immediate infiltration of leukocytes and fibroblasts between muscle bundles and fibers, followed by rapid necrosis which was manifested by loss of nuclei and striations in some bundles within one week.

Mononucleated myoblasts were formed and some regeneration was seen but differentiated fibers did not reappear. By six weeks muscle degeneration and fibroblast penetration had spread beyond the area of injection. Between eight and fourteen weeks myoblasts became large and multinucleated, with many mitotic figures apparent, and giant cells were seen. The tumour nodule did not become palpable until twenty weeks at which time the cobalt was still present. Heath postulated that cobalt was capable of producing muscle tumours by virtue of a modification of normal regenerative and repair processes due to the presence of the metal. Cobalt ions were probably liberated continuously by slow solution in tissue fluids or the muscle cells may have been subjected to direct catalysis at the surface of the metal grains.

It would seem that the continued presence of a metal is a prerequisite for production of tumours. The experimental work on Ni_3S_2 and NiS has indicated that, in the majority of cases, nickel particles were present in rats which developed tumours while those rats in which the nickel had disappeared showed no evidence of muscle abnormality. Gilman has recently reported the subcutaneous implantation of discs of nickel sulphide rather than injection of the metal as a particulate suspension (30). This might be a more reliable method of

ensuring a small but constant supply of nickel ions. He has noted the presence of myoblast cells after eight weeks of contact with the metal, but states that transplantation of similar tissue is not effective until after eleven weeks of exposure. Tumours do not become palpable until after sixteen weeks. Such results indicate a critical exposure period of muscle to nickel sulphide.

There appears to be no obvious explanation why NiS failed to remain in most of the injected muscles. The question of the importance of particle size has not been answered. It is conceivable that if the injection volume contained particles small enough to be in a colloidal form, these could possibly diffuse quickly out of the muscle, causing systemic toxicity and no muscle tumours. This would indicate that it is necessary for a critical amount to remain in the muscle region. However, the particles of both compounds tested were of a similar size and were not soluble in the injection vehicles, thus both would presumably be located within the muscle as a particulate mass. Their further fate is entirely unknown. It is possible that only NiS was rapidly excreted by the kidney but again there seems no rationale for such a statement. Studies on the comparative excretion rates of the two compounds are planned

for future experiments in this laboratory. The possibility of different solubilities in vivo, as indicated by urinary analysis, is discussed in greater detail below. The fact that the continuing quantitative mass of the metal may be important suggests that its detection in the urine might indicate differences in behaviour between the two forms of nickel sulphide.

An alternative possibility to that of particulate mass affecting stability of the compound is that direct contact with muscle acids may have some effect. Once interspersed among the muscle bundles, nickel sulphide would undoubtedly be exposed to a relatively higher acidity, due to lactic and pyruvic acids. Whether the two compounds could have different solubility properties in muscle acids has not been determined, although both compounds were found to have similar solubilities in hydrochloric acid. If NiS were less stable in acid and were situated deep within the belly of the muscle it might be subjected to a greater amount of acid capable of dissolving it away. This hypothesis is tentatively suggested by the fact that the tumour arising from NiS arose at the upper external surface of the gastrocnemius and appeared to be connected to it rather than embedded in it.

If lactic acid does have an effect on nickel sulphide, this same acid environment might also play some role in the rapidity of tumour induction in striated muscle. Lactic acid is well known as a common metabolic by-product of rapidly-growing tumours and has been implicated in their invasive characteristics by the speculation that the release of free acid and decrease in pH might aid the destructive action of tumour cells adjoining normal tissues. However, the amount liberated is probably quickly converted by the liver to glycogen. An opposing theory has cited that lactic acid will diminish the carcinogenic potency of some substances (84). Thus, the true role of this acid remains an ambiguity in the neoplastic processes.

To have any carcinogenic effect on voluntary muscle an agent would have to exert a strong effect on this differentiated tissue in order to make it malignant. The question of whether mature striated muscle fibers are capable of mitotic division has been open to some discussion. Swann has stated the negative point of view emphatically by remarking that every tissue in the body of a higher animal shows at least occasional mitoses, with the exceptions of nerve cells and striated muscle (79). Thus even though most differentiated cells are capable of dividing, this ability is lost irretrievably

in striated muscle. The case has been presented less firmly by Willis in his description of the limited capacity of striated muscle for proliferation once it has attained adulthood (84). A more thorough analysis of the problem has been given by Adams et al. in Diseases of Muscle (1). During the embryological development of the muscle, mitotic division is thought to cease after the fifth month in humans and further enlargement in muscles should be by an increase in the size of the individual fibers rather than in the number of fibers. In referring to the mature fiber, the statement is made that in view of the embryonic origin of striated muscle fibers from myoblasts, which have formed by mitotic division but which then become multinucleated syncytiums by amitosis, it is probable that mitotic division would occur only if the muscle fiber reverted to a primitive unicellular or myoblast state.

This could be correlated with Heath's study mentioned previously on the effects of a metallic ion on striated muscle. The original response of the muscle to the mechanical injury of trauma produced by injection of the metal particles would be an attempt at regeneration and repair. However, if there were continued action of the metallic irritant, the muscle might fail to complete the regenerative processes and instead

continue to dedifferentiate, being reduced eventually to the embryonic myoblast state. Once at this level, ensuing mitotic figures might be altered directly by the nickel ions, such that the final result would be a malignant state.

This does not explain, of course, how nickel sulphide in particular would alter the muscle cells or why it should have any specificity for striated muscle. Presumably the tissue reaction to nickel would be more than just the response to a foreign irritant since nickel is not an inert substance. Considered to be the commonest specific skin sensitizer, its action in dermatitis is probably the formation of protein complexes through linkages with sulfhydryl groups of amino acids (31, 47). Hueper has advanced a theory that nickel may inhibit the action of free sulfhydryl groups and cause enzymatic disturbances which lead to cancer (47). A similar action has been tentatively attributed to metals in general by Haddow who suggested that introduction of excess metal into a carefully balanced system of metals and metal enzymes could result in interference with certain functions such as tissue respiration, thus causing cellular mutation (35). However, this theory is merely speculative, especially in application to nickel, since the presence of this metal in normal body tissues is not vital.

The great variability observed in growth characteristics of the different rhabdomyosarcomas was not unexpected since the cell populations which made up different transplant lines would acquire specific behaviour patterns when they first originated. In most cases, growth rates and amount of dedifferentiation did not change during transplant generations. The stability of individual characteristics which tumours exhibit is one of their most remarkable properties and shows that neoplasia must result from an irreversible, hereditable change in the cells themselves (84). Such changes in response as did occur after repeated serial transplants served to exhibit the phenomenon of progression. Foulds has stated that progression depends more on intrinsic properties of individual tumours than on the environment to which they are all exposed, and transplantation may merely complete a progression whose course was set in the primary tumour (25). The tumour may continue to become more unresponsive to treatments in each generation, although maintaining similar growth characteristics. Thus any experimental work on transplants must allow for such variations.

The desirability of having a reliable tumour for use as a test system for therapeutic compounds is considered to be extremely important in cancer research. In employing solid transplanted tumours for experimental purposes, certain criteria

for evaluation of effect had to be set up, as well as regimens for administration of test compounds. The latter problem depended on empirical choice of route, frequency, dose level and time at which treatment should be initiated. In evaluating the effect, the criterion for a positive reaction had to be definitely stated in order to avoid confusion as to the comparable effectiveness of particular compounds.

In the case of the Vinca alkaloids, inhibition of tumour growth within a certain time period, as determined by measurements and calculated weights, was the desired endpoint. Dose regimens were chosen empirically with regard to amounts administered while the choice of different periods of injection was made in order to see what effect the establishment of the tumour had on its susceptibility to treatment. With Vinblastine there was little difference in tumour growth when the compound was given after the tumour had become well-established. However, some of the test animals did not develop tumours throughout the entire test period when Vinblastine was administered during the first week. With Vincristine, also, the response seemed to be somewhat better when treatment was given during the early stages of tumour establishment.

The mechanism of action of both alkaloids is unknown, although anti-tumour effects have been well documented in

several animal experiments (51). In most cases the response has been of two types, as seen in the present experiments. The most striking effect is suppression of takes, with indefinate survival of the treated animals, while the more common response is inhibition of growth resulting in prolonged but limited survival. The effect of early treatment with the alkaloids on percentage of tumour takes has been observed particularly on P1534 leukemia in mice, where initiation of treatment six days after implantation results in absence of takes and indefinate survival. It is thought that the striking control of transplant growth might be due to some inhibition of the vascular supply which is necessary for any tumour development.

Both Vinblastine and Vincristine are capable of causing mitotic arrest in both in vivo and in vitro systems (16, 51). The specific effect is thought to be on the spindle so that the chromosomes are held at metaphase, presenting a picture known as C-mitosis. Cutts showed that a single intraperitoneal injection of Vinblastine to animals bearing tumours caused an increase in the number of cells in metaphase, while multiple doses at daily intervals had an additive effect on accumulation of metaphases (16). However, Johnson, in comparing this

activity with that of a known spindle inhibitor such as colchicine, has stated that the similarity is probably coincidental and does not advance it as an explanation of the anti-tumour action (51). Further biochemical studies have suggested a possible anti-metabolic effect involving amino acids, but as yet no accepted theory has been presented for the mode of action of the Vinca alkaloids.

The testing of experimental tumours with hormones has a double rationale. The more general reason is to record any new tumour, regardless of morphology, which responds non-specifically to various hormones. Certain adrenocortical compounds have been used in cancer therapy with varying degrees of success, depending on the tumour systems tested. They have often been employed as specific antimitotic or antiproliferative agents (18). A more specific purpose is to investigate the possibility that the tumour may be hormone-responsive, by virtue of its tissue of origin. In the case of certain steroids, derived from the adrenal cortex and the testes, a study of their effects on tumours of striated muscle was deemed particularly interesting in view of their physiologic effects on protein metabolism.

Voluntary muscle is known to exhibit completely opposite reactions, with respect to its protein metabolism,

after administration of adrenal glucocorticoids on the one hand and androgens on the other. The effects of glucocorticoids have been demonstrated to be severe depletion of tissue protein and a negative nitrogen balance, manifested by muscle atrophy and weight loss. Conversely, the administration of androgens results in a myotrophic or protein anabolic effect, causing generalized muscular hypertrophy. It seemed logical that a muscle tumour might also exhibit these antagonistic effects in response to exogenous corticosteroids.

At the present time there is only one physiological system in use for large scale testing of testosterone and androgenic derivatives. It involves the myotrophic action of such compounds on the levator ani muscle in rats. After injection with appropriate compounds, the muscle is dissected out and its weight is compared with controls, a twofold increase in size being indicative of active anabolic effects. It was thought that a responsive rhabdomyosarcoma might provide another test system for such compounds.

The use of a wide variety of corticosteroid dose regimens in attempted treatment of rhabdomyosarcomas was necessitated by the lack of other experimental information on such tumours. All routes and doses chosen were pharmacological since the rat has no endogenous secretion of cortisol or cortisone.

The only constant factor was the time of initiation of treatment, on the seventh day after transplantation. This time lapse was allowed in order for the tumour graft to achieve vascularization. It is an established fact now that most transplanted tumour cells acquire a blood supply from the host. Algire and Chalkley showed that the vascular system of a tumour owes its origin to the host's response to the tumour transplant (3). Immediately following transplantation, fluid and leukocytes accumulate around the foreign cells and adjacent capillaries begin to dilate during the next few days. By the fifth to seventh day definite vessels are present in the tumour, occupying a stabilized 40 to 50 per cent of the total tumour volume (2). Concomitant with this penetration, the rate of growth of the tumour becomes approximately constant and gross tumour growth is now evident. The vascular supply of the tumour is usually 100 per cent greater than that of the surrounding connective tissue, indicating that the tumour will have the preferred nutritional status of the two tissues. Since this same phenomenon occurs, although only temporarily, in wound healing, it is thought that the tumour merely appropriates the host vessels which have proliferated as they would in response to any foreign body or wound.

The tumour vessels are generally similar to capillaries in structure, having only a single endothelial layer, but the diameters are often large. Merwin and Hill have described the enlarging and convoluting of the host capillaries (62). The resultant tortuosity and slow blood flow may be significant in the possible sensitivity of transplanted neoplasms to anti-tumour compounds. Since the expected poor oxidation and accumulation of lactic acid might affect subsequent growth of rhabdomyosarcomas, initial vascularization was considered to be an important and necessary factor before treatment was initiated.

Despite the variability of results obtained with corticoid administration, certain interesting trends were indicated. The first of these was the startling inhibition of growth, without attendant weight loss, resulting from intra-peritoneal injections of either cortisol or cortisone to an early transplant generation of one tumour. Radioactive tracer studies have shown that intraperitoneally administered cortico-steroids will reach a peak in the blood stream within half an hour and be completely removed after two hours (13). The lack of protein breakdown, as shown by weight loss, following this route of administration was probably due to the fact that there was little time for the steroids to act on the body's protein reserves. The repeated attacks of high doses might explain

the anti-tumour action, while the rapid disappearance of the injected material might result in the inhibition of growth only during the injection period.

The curious phenomenon noted when a higher dose of intraperitoneally administered cortisol proved ineffective against a tumour line which was originally very susceptible served to emphasize the main drawback in using transplantable tumours as test systems for therapeutic compounds. Snell has stated that tumours may change during many transfers due to morphologic or physiologic alterations (74). In the former case, responsive normal cells which might originally have been carried along with the tumour have now become neoplastic or mutations within tumour cells may have taken place. Physiologic changes in the tumour may include an increase in the number of faster-growing cells or an enhanced ability to kill hosts which were previously resistant. The fact that the histological composition of the tumour had not changed appreciably indicated true progression, where one characteristic, hormone sensitivity, had changed independently of growth.

The adoption of subcutaneous injections was decided upon in order to determine any effects of a more prolonged presence of corticoids. Such compounds injected in this manner should be released slowly from the site of injection,

accumulating in a depot for a few days. Therefore one would suppose that the effective dose should rise slightly at the start of the injection period and fall off slowly at the end. The fact that the tumour growth rate was inhibited only partially during the time of treatment indicated that the steroids probably had a slower but more prolonged effect. The weight loss resulting from this treatment was not unexpected, since the continued presence of cortisol or cortisone would logically result in continued breakdown of the protein reserves of the body.

Another interesting point was illustrated by the combination treatment of one tumour with both cortisol and testosterone. This was done in order to determine if the decreased weight of the test tumours was a result of general systemic weight loss. The fact that tumour weight was inhibited in animals treated either with cortisol or with cortisol plus testosterone, while the body weight loss was reversed in those receiving both hormones, gave clear-cut evidence that the anti-tumour effect of cortisol was distinct from its catabolic effects in rhabdomyosarcoma-bearing animals.

Treatment of some test rats with testosterone showed rather equivocal effects. In an ideal situation, the tumours should

have been slower growing so that any increase in protein anabolism would be more obvious. However, some tentative indications of action could be noted. Although there was no effect if the hormone was administered after the first week of growth, some examples of accelerated growth were seen when testosterone was injected twenty-four hours after transplantation. This suggested a mode of action different from that of other steroids, with this hormone's effect being mediated by some means other than established vascularization. A possible explanation is that testosterone may have been exerting its myotrophic effect on muscle elements still present within the rhabdomyosarcoma. On this basis, however, one would expect those transplant generations closest to the primary tumour to show the greatest response, since they would retain a greater amount of original tissue characteristics. This was obviously not the case, with both sensitive lines being in the eleventh generation.

The anti-tumour actions of the adrenal steroids are relatively unknown, although it has been suggested that corticoids may interfere with growth of certain mesodermal structures and connective tissue, attacking such elements as fibroblast growth (53). Similarly, the action of testosterone

has not been demonstrated to satisfaction, other than by the gross physiologic response of hormone dependency in certain tumours derived from endocrine tissues (68). Thus the divergent responses obtained in the present studies do not necessarily mean that the experiments have had essentially negative results. In a search for sensitive and resistant animal strains to serve as reliable test systems, any information which results from empirical testing of new tumours is of some benefit. Because of the variability of response, transplantable rat rhabdomyosarcomas would probably not be of great value for routine testing of steroid compounds.

CONCLUSIONS

A study of the induction of rhabdomyosarcomas in rats by the injection of nickel sulphide provided information not only on the carcinogenic activity of nickel sulphide but also on the growth and development of rhabdomyosarcomas in rats.

It was found that intramuscular injections of Ni_3S_2 resulted in fairly rapid appearance of tumours at the site of injection, with 20 mg. causing 100 per cent tumours after four months. These tumours were examined by histological means and their identity established as rhabdomyosarcomas. After their first appearance as swelling of the muscle region, they grew rapidly, resulting in complete atrophy of the injected muscle.

On transplantation the tumours proved to be highly malignant, with the average host mortality ranging between three and eight weeks. Attempts at experimental tumour therapy proved rather equivocal. Tests with the Vinca alkaloids seemed to be most effective in causing inhibition of growth when both Vinblastine and Vincristine were administered during the first week after implantation. Administration of steroids for anti-tumour and metabolic experiments resulted in a response ranging from residual to complete inhibition of many tumours following

corticoid treatment, and acceleration of growth of some tumours following testosterone administration. In general the tumours appeared to be essentially unreliable for such studies due to unpredictable changes in response among different generations.

Preliminary experiments on the carcinogenicity of NiS suggested that it was more toxic and less carcinogenic than Ni_3S_2 under similar experimental conditions. A possible reason for this difference in activity may have been the rapid disappearance of NiS from the injected muscle areas, noted in most animals.

The possible carcinogenicity of different metal salts offers many avenues of further experimentation. The problem of solubility in muscle acids can be examined from two directions. First, the intramuscular or subcutaneous implantation of weighed metal pellets would provide a continuous supply of nickel to the muscle, while their later removal and weighing would show exactly how much had been released. In addition, the solubilities of closely related compounds such as Ni_3S_2 and NiS could be compared by estimations of the amount of nickel excreted in the urine. The alleged specificity of nickel sulphide for striated muscle might be validated by more extensive experiments involving exposure of several body

tissues to the compounds for longer periods of time.

The rapid induction of rhabdomyosarcomas by nickel sulphide presents many opportunities for the investigation of early, premalignant changes, since the histogenesis of such tumours is relatively unknown. Serial studies of the exposed muscle would provide information on its acute and chronic reactions to foreign irritants, followed by possible tissue damage and neoplasia. Periodic biopsies of the muscle and, more particularly, the developing tumour, might give some indication as to whether the tissue undergoes progressive changes to an ultimate malignant form. The comparative rarity of this tumour offers a challenging experimental model for research purposes.

APPENDIX

Mallory's Phosphotungstic-acid Haematoxylin (PTAH) Stain:

1. Remove wax and bring sections to water
2. Mordant sections in mercuric chloride for three hours at room temperature
3. Rinse in distilled water
4. Stain with Lugol's iodine, fifteen minutes
5. Rinse in two changes of 95 per cent alcohol
6. Rinse in distilled water
7. Stain with 0.25 per cent potassium permanganate, fifteen minutes
8. Rinse in distilled water
9. Stain with five per cent oxalic acid until bleached white
10. Rinse in distilled water
11. Stain with Mallory's PTAH, eighteen to twenty-four hours
12. Rinse in 95 per cent alcohol
13. Dehydrate, clear and mount

Perl's Prussian Blue stain:

1. Remove wax and bring sections to water
2. Mix equal amounts of freshly made 2 per cent potassium ferrocyanide and 2 per cent hydrochloric acid; stain sections one hour
3. Rinse in distilled water
4. Counterstain with eosin, two minutes
5. Dehydrate, clear and mount

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