Results. Table I presents the effects of T-3 at three dose levels on liver PPh activity and its inactivation upon incubation in the absence of fluoride.

T-3 administration resulted in a significant decrease in the liver PPh activity. From the above results, there appears to be some relationship between the amount of T-3 administered and the decrease in liver PPh observed.

When liver homogenates are incubated at 37°C with an equal volume of 0.02 *M* Tris buffer (pH 7.4) a very rapid fall in PPh activity occurs. IE, which in the absence of fluoride promotes such an *in vitro* inactivation, appears to be greatly stimulated in liver homogenates from T-3 treated rats.

A slower but eventually significant fall in liver PPh activity was observed also when the homogenates were incubated at 37°C with Tris buffer (pH 7.4) in the presence of 0.1 *M* KF. Since fluoride at this final concentration effectively inhibits IE activity, the decrease in PPh could be attributed to proteolitic degradation of the enzyme molecule (Table II).

The results listed in Table II indicate that T-3, even when administered in relatively small doses (50 µg/kg body weight), markedly stimulates the inactivation of PPh which takes place in the presence of 0.1 *M* fluoride.

Discussion. The present studies indicate that T-3 administration induces a decrease in rat liver PPh activity and stimulates its *in vitro* inactivation due both to IE and to proteolitic degradation.

A simple and attractive interpretation of the above results would be that the decrease in liver PPh activity is dependent on an increased IE. In this case a greater transformation of Phosphorylase into the inactive *b* form could explain the fall in PPh activity. However, recent experiments in our laboratory indicate that, in the liver of the control and T-3 treated rats, Phosphorylase exists predominantly in the *a* form. Moreover, the administration of epinephrine (which stimulates Phosphorylase *a* formation) did not restore the decreased liver PPh activity after T-3 treatment. This would indicate that in intact animals the activation of the enzyme greatly prevails over the inactivation, and that IE activity, even when stimulated after T-3 treatment, cannot elevate the low Phosphorylase *b* concentration.

The above considerations could mean that the decrease of PPh is dependent on a low concentration of the enzyme itself in the hepatic tissue. This conclusion would indicate that T-3 administration can inhibit the biosynthesis of the enzyme or stimulate its proteolitic splitting. The lowering of ATP content[5,6] could explain a decreased biosynthesis of the enzyme, whereas the results listed in Table II are consistent with an increased breakdown.

Table I. PPh and IE activities in liver homogenates from control and T-3 treated rats

Group	No. of rats	Daily treatment	Liver PPh (mg Pi/g/8 min) before incubation	Liver PPh (mg Pi/g/8 min) after incubation	IE
I	6	None	10.5 ± 0.54	7.5 ± 0.42	3.0
	6	T-3 (500 µg/kg)	6.8 ± 0.66	1.7 ± 0.28	5.1
II	6	None	10.8 ± 0.48	7.7 ± 0.60	3.1
	6	T-3 (100 µg/kg)	7.5 ± 0.62	2.7 ± 0.35	4.8
III	6	None	11.2 ± 0.50	8.4 ± 0.44	2.8
	6	T-3 (50 µg/kg)	8.7 ± 0.56	4.3 ± 0.32	4.4

Liver homogenates were diluted with an equal volume of 0.02 *M* Tris buffer (pH 7.4); PPh activity was determined before and after 4 min incubation at 37°C. IE was estimated by the decrease in PPh activity upon incubation at 37°C in the absence of fluoride. Average control and T-3 treated values are given ± SE.

Table II. Inactivation of liver PPh from normal and T-3 (50 µg/kg) treated rats in the presence of fluoride

Treatment	No. of rats	Liver PPh (mg Pi/g/8 min) before incubation	Liver PPh (mg Pi/g/8 min) after incubation	Inactivation
None	6	11.2 ± 0.5	9.1 ± 0.6	2.1
T-3	6	8.7 ± 0.5	5.4 ± 0.7	3.3

Liver homogenates were diluted with an equal volume of 0.02 *M* Tris buffer (pH 7.4) containing 0.2 *M* KF; PPh activity was determined before and after 10 min incubation at 37°C. Average control and T-3 treated values are given ± SE.

Riassunto. L'attività polisaccaride fosforilasica presente negli omogenati di fegato dei ratti trattati per 3 giorni con T-3 è sensibilmente diminuita; sono invece nettamente aumentate, in assenza di fluoruro, la trasformazione della forma *a* in *b* e, in presenza di fluoruro, l'inattivazione irreversibile dell'enzima. Nel fegato degli animali ipertiroidei la diminuzione dell'attività polisaccaride fosforilasica non sarebbe però dovuta all'esaltata inattivazione, ma alla minore concentrazione dell'enzima stesso.

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Istituto di Fisiologia Generale dell'Università di Genova (Italy), June 12, 1963.

Induction of Tumors in Rats by Subcutaneous Implants of Surgical Sponges[1]

It has been demonstrated repeatedly that experimental subcutaneous implantation of a wide variety of plastics and other substances, especially in the form of films, frequently gives rise to neoplasms[2–4]. Nevertheless, it appears to be widely assumed that polyvinyl alcohol surgical sponges (Ivalon), when implanted into living animals, are relatively inert, are well tolerated by adjacent tissues, and are without carcinogenic activity[5]. Most unfavorable reports on the use of Ivalon sponges as prostheses have found them to be unsatisfactory for reasons other than for non-tolerance or carcinogenicity[6].

On the basis of previously unpublished microscopic observations by MILLISER, we have long felt that polyvinyl sponge implants are not as inert as is generally supposed. Giant cells have been observed in all implants after the first few days. Erosion of the sponge substance (lysis? phagocytosis?) has seemed to take place and has appeared to be more prominent in the older implants. We therefore decided to examine sponge biopsies after long periods of implantation.

Male Holtzman rats, 5–6 weeks of age and weighing 150–200 g, were each implanted in June, 1960, with 2 circular polyvinyl alcohol sponges (Ivalon), 20 mm in diameter and 3–4 mm thick. The technique of implantation was the same as that previously described[7] except

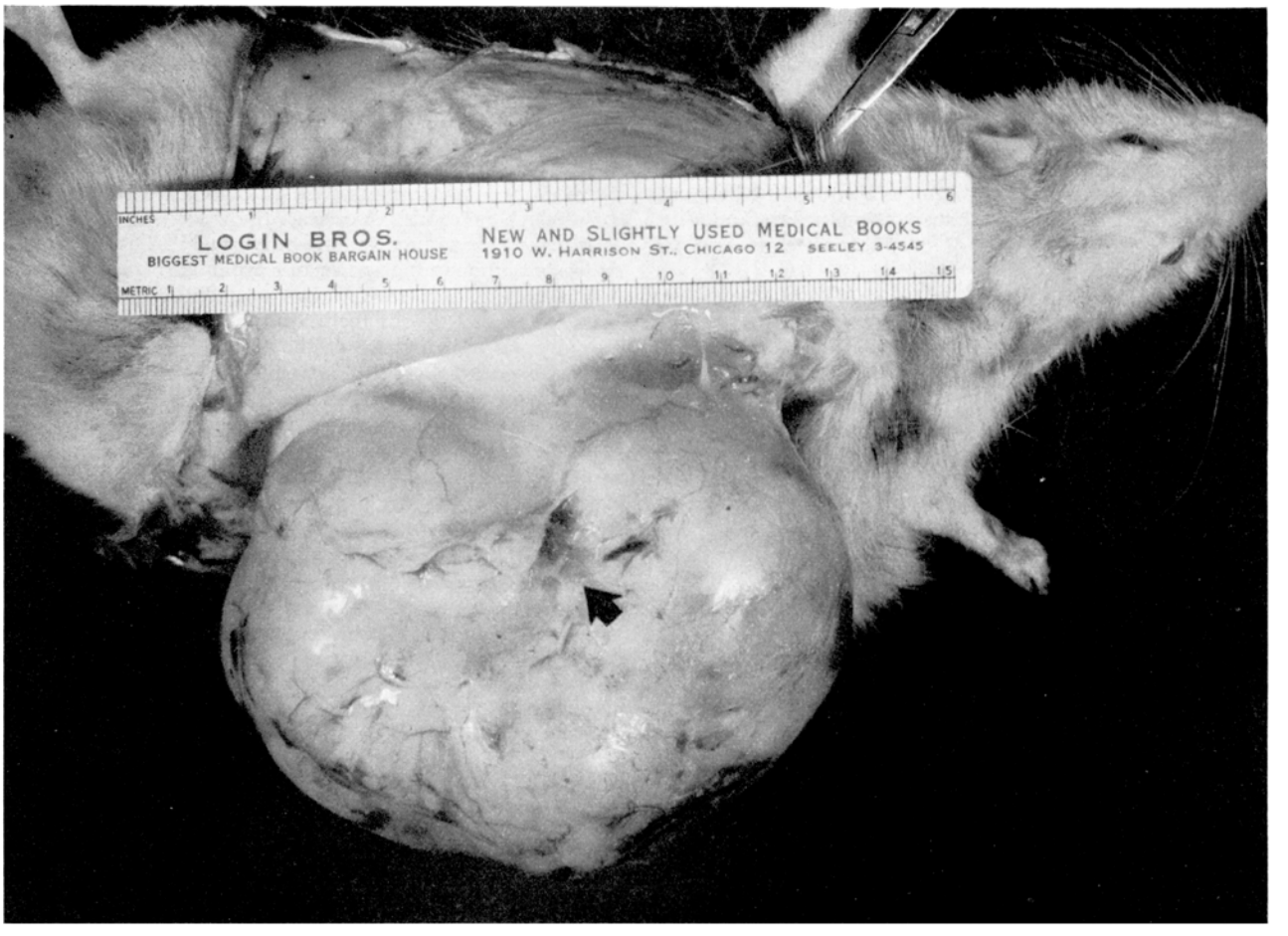

Fig. 1. Rapid-growing tumor. Arrow indicates location of the sponge which was completely embedded in, but at the periphery of, the tumor.

that no tap water was used and thorough preliminary rinsings of all sponges were carried out with distilled water alone.

During the first 17–18 months following implantation, a number of animals were used for various purposes and an additional number died from undetermined causes. On December 7, 1961, an examination of the 12 surviving animals revealed 5 rats with tumors involving 1 of the 2 implanted sponges and 7 apparently normal animals. One normal control and 3 animals with tumors were sacrificed at this time. Of the remaining 6 normal (i.e., non-tumorous) animals, 2 died within 2 days of unknown causes while the remaining 4 all developed palpable tumors within the next 4 months. Thus, of the 12 rats first examined for tumors about 18 months after implantation of the sponges, 9 either had tumors or developed tumors later. This represents a *per rat* incidence of 75% based on the number of survivors when first examined. The *per sponge* incidence of tumors was 42% since only 1 animal was found to have tumors involving both of its implanted sponges. Since all animals which survived as long as 4 months following the first inspection of 12/7/61 eventually developed tumors, it is reasonable to assume that a higher survival rate would have increased the incidence of tumors even more.

Rate of tumor growth varied widely, from some that remained almond-sized and seemed not to grow over a period of months, to one whose rate of growth increased rapidly to an average weight gain of 21 g/day during the last 4 days before sacrifice. The latter, when removed, measured 13 × 9 × 7.5 cm and weighed 459 g (Figure 1), the intact rat with its tumor weighing 918 g. In 8 of the 9

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tumors the sponges were completely or partially embedded in the tumor. In 1 case the sponge lay completely outside, but on the periphery of, a large tumor weighing 209 g.

All tumors appeared to be fibrosarcomas (Figure 2). No metastases were found in any of the animals, but local invasion and infiltration of adjacent tissues were evident in all. Attempts to transmit tumors to normal rats were made by injecting tumor cell suspensions subcutaneously and intraperitoneally. Out of 34 subcutaneous and 10 intraperitoneal injections only 1 'take' occurred. An intra-abdominal tumor became noticeable about 5 months after 1 of the intraperitoneal injections. About 2 weeks later this rat died with a tumor weighing 341 g.

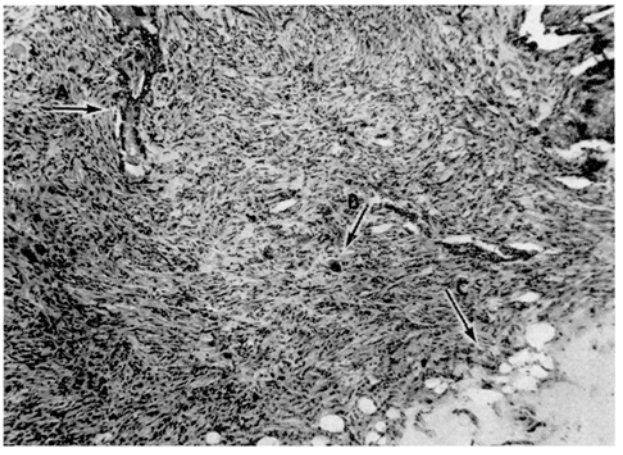

Fig. 2. Section through periphery of tumor showing sponge trabeculae (A), multinucleated giant cells (B), and invasion of adjacent adipose tissue (C). (× about 350 before reduction)

OPPENHEIMER et al.[3] reported a tumor incidence of 8.8% resulting from the subcutaneous implantation of polyvinyl alcohol (Ivalon) sponges in rats. In subsequent papers[4] they state that when plastics are embedded in forms 'such as textiles, sponges, or powders' (as contrasted to films), 'they induce tumors only rarely'. The high incidence of tumor induction in our animals may have been due in part to the strain of rat used, but may also have been influenced by our technique: (1) sponges were sterilized by autoclaving rather than by boiling, (2) sponges for implantation were wet with distilled water rather than with normal saline, (3) the screw caps of the glass vials in which the sponges were autoclaved had Vinylite liners. It is unlikely, however, that any of the details of technique would have more than a transitory effect on the tissues involved, whereas it takes almost 18 months for the first sponge-induced tumors to make their appearance.

Zusammenfassung. Polyvinylalkoholschwämme, in Tiergewebe eingepflanzt, sind weniger inaktiv als allgemein angenommen wird. Fibrosarkome entstanden in 75% der Ratten, welche die subkutane Schwammeinpflanzung mindestens 18 Monate überlebten.

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Androgenic and Anabolic Hormones: A new Group of Substances Stimulating Endogenous Ascorbic Acid Synthesis in the Rat

It is a well known fact that some animals are capable of synthetising ascorbic acid in their organism while others lack this quality. There are only few examples of the latter variety, viz. man, monkey, the Indian fruit bat and the guinea-pig, which cannot produce this vitamin[1].

Various drugs possessing completely unrelated chemical and pharmacological properties have been shown to enhance markedly the urinary excretion of L-ascorbic acid when administered to animals having the property of ascorbic acid synthesis. The following drugs belong to this group: Chloretone and barbital, aminopyrine and antipyrine, diphenhydramine and chlorcyclizine, 3-methylcholanthrene and 3,4-benzpyrene, orphenadrine and meprobamate, SKF 525 A and various substances having carcinogenic or toxic effect on the liver[2-4]. It is known that these substances have an effect on specific liver enzymes which are intimately connected in the synthesis of endogenous ascorbic acid. Chloretone, for instance, has been shown to accelerate the conversion of D-glucose to D-glucuronic acid *via* the oxidation of uridinediphosphate-D-glucose to uridinediphosphate-D-glucuronate by stabilizing UDPG dehydrogenase[5]. Growth hormone also stimulates endogenous ascorbic acid synthesis in rats[6]. This effect may be mediated through an increase in the biosynthesis of the specific liver enzymes.

Androgenic and anabolic hormones have a growth-promoting property like growth hormone. These hormones have also an increasing effect on amino acid activating enzymes[7]. The present investigation was undertaken to study the effect of these hormones on ascorbic acid synthesis.

The experiments were carried out by using female and male rats of Long–Evans strain. Both young, 2 months old, and adult, 4 months old rats were used with the average weight of 120 g and 200 g respectively at the beginning of the experiment. The animals were maintained on a standard diet chow and received water *ad libitum*. The young rats were divided into groups of 5 rats with equal group weight at the beginning. The adult rats were divided into groups of 3 rats with group weight averaging the young rat groups.

The following substances were injected subcutaneously in the rats: (1) testosterone propionate (Neo-Hombreol®) 10 mg/kg and 20 mg/kg; (2) 17β-hydroxy-19-norandrost-4-en-3-one-17 phenylpropionate (Durabolin®, abbreviated nor-TPP) 1.25 mg/kg, 2.5 mg/kg and 5.0 mg/kg; (3) 17β-hydroxy-19-norandrost-4-en-3-one-17 decanoate

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