Schuster and Schramm(3) concluded that the deamination of 2 groups was, in average, lethal. It thus does seem probable that all RNA inactivating reactions need involve but a small fraction of the potentially reactive sites. The ability to coaggregate with native protein may not be noticeably affected by this extent of modification of the RNA structure.

It seems possible that these observations may have future practical implications. When the nucleic acid free proteins of pathogenic viruses will have been isolated in native form, these may prove more suitable for immunization than the "killed" viruses. If, however, they should prove less than perfect, because they lack the typical virus achitecture‡, then reconstitution of such a native protein with the isolated and thoroughly inactivated preparation of the corresponding RNA might supply the most effective means of safe immunization. Unfortunately, reconstitution has yet been possible only with TMV and its strains.

Summary. TMV-RNA inactivated by various means, in particular by reaction with propylene oxide, retains its ability to form virus-like rods with native TMV-protein. Such reconstituted inactive virus is serologically indistinguishable from the intact virus. The structural and potential practical implications of this observation are discussed.

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Correlation between Oxygen Consumption and Erythropoiesis in Hypophysectomized Rats Treated with Various Doses of Thyroxin.* (25164)

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Anemia which develops in hypophysectomized animals is accompanied by a decrease in oxygen consumption(1-4). Therapies which have eliminated this anemia, such as a combination of thyroxin, cortisone and growth hormone(3,4), have elevated oxygen consumption. It has been proposed(1,2) that anemia in the hypophysectomized rat is an expression of a decreased need for oxygen of the animal, and that the above therapy proved beneficial because of increased oxygen need resulting from such treatment. This was tested by altering oxygen consumption with various doses of thyroxin given in conjunction with cortisone and growth hormone(4). Oxygen consumption and erythropoiesis varied directly with the dose of thyroxin given. Several investigators studied the effect of thyroxin on hypophysectomized rats(5-8). Partial alleviation of anemia, bone marrow repair and reticulocytosis have all been reported but no attempts were made to relate the effect of various doses of thyroxin to changes in oxygen consumption and alterations in erythropoiesis. If the oxygen need theory is correct, thyroxin

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TABLE I. Effects of Hypophysectomy Alone and Hypophysectomy Followed by Treatment with Varying Doses of Thyroxin on Oxygen Consumption and Total Erythrocyte Volume in Adult Female Sprague-Dawley rats. ± = stand. error.

	Normal controls	Hyp., no treatment		Hyp., 0.01 mg thy./day × 45		Hyp., 0.05 mg thy./day × 25		Hyp., 0.10 mg thy./day × 10	
	32 rats	23 rats	% of normal	14 rats	% of normal	16 rats	% of normal	8 rats	% of normal
Body wt, g	267 ±2.80	213 ±3.73	80	191 ±2.50	72	163 ±2.57	61	163 ±4.8	61
Oxygen consumption, l/m² body surface/hr	9.01 ±.14	6.06 ±.20	67	9.02 ±.25	100	11.17 ±.20	124	9.61 ±.36	107
Plasma vol, cc	8.15 ±.14	6.63 ±.09	81	5.48 ±.07	67	5.96 ±.19	73	5.70 ±.34	70
Blood " "	14.98 ±.20	10.96 ±.14	73	9.54 ±.14	64	11.01 ±.30	73	10.18 ±.55	68
Total erythrocyte vol, cc	6.83 ±.12	4.33 ±.07	63	4.06 ±.08	59	5.05 ±.14	74	4.48 ±.24	66
Idem, per 100 g body wt	2.56 ±.06	2.04 ±.04	80	2.13 ±.05	83	3.11 ±.10	121	2.74 ±.11	107

alone, which increases oxygen need, should stimulate erythropoiesis and one should be able to obtain a variation in erythropoiesis with varying doses of thyroxin in the absence of cortisone and growth hormone. This experiment was devised to determine the effects of various doses of thyroxin on oxygen consumption, and to see whether erythropoiesis would mimic any changes elicited.

Materials and methods. Female rats, 3 to 4 months of age, of Sprague-Dawley (Holtzman) strain were fed standard Purina chow *ad lib.* supplemented once a week with lettuce. Completeness of hypophysectomy was checked at autopsy by examining the organ site with a dissecting microscope. Blood volume measurements were made by the Evans Blue dye method as described by Metcoff and Favour (9). The method used for determining oxygen consumption has been described(1). Bone marrows were saved for histological study. Femurs were removed at autopsy, the ends cut off and one side of shaft cut away. The remaining bone and marrow was placed in Helly fixative, later washed and placed in 70% alcohol. Subsequently the marrow was removed from the bone in a long strip, cleared in dioxane, embedded, sectioned at 6 μ, and stained with Giemsa stain.

Procedure. Ninety-three rats were used; 32 served as normal controls and 61 were hypophysectomized. After the hypophysectomized rats became anemic, they were divided into 4 groups as follows: (1) no treatment, (2) daily subcutaneous injections of 0.01 mg thyroxin,‡ (3) daily subcutaneous injections of 0.05 mg thyroxin, and (4) daily subcutaneous injections of 0.10 mg thyroxin. These doses were chosen because these were the same as given in combination with cortisone and growth hormone(4). Although it was planned to inject all treated groups the same number of days, it was soon apparent that the higher doses were causing a severe weight loss. Accordingly, those receiving 0.01 mg daily were injected for 45 days, those receiving 0.05 mg for 25 days, and those receiving 0.10 mg only 10 days. In spite of this discrepancy in time, the data proved interesting.

Results are summarized in Table I. Animals hypophysectomized and not treated exhibited a 33% decrease in oxygen consumption, accompanied by a 21% decrease in total erythrocyte volume/100 g body weight. Treatment with 0.01, 0.05 and 0.10 mg of thyroxin raised oxygen consumption of the hypophysectomized rats to 100, 124 and 107% of normal respectively; these elevations were accompanied by erythrocyte volumes/100 g body weight 83, 121 and 107% of normal.

Careful study of histological sections of bone marrows (Fig. 1-5) substantiate these results. Hypophysectomy induced the usual

‡ DL thyroxin.

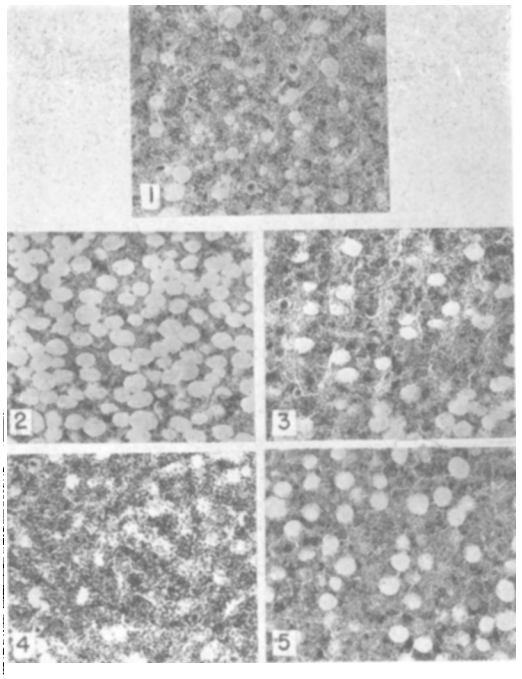

FIG. 1. Femur marrow from normal rat 5 months of age (× 80). Note amount of fat and islands of dark cells representing erythroid elements.

FIG. 2. Femur marrow from hypophysectomized animal 4 months after surgery (× 80). Note increase in amount of fat and decrease in size and distinctness of islands of erythroid elements.

FIG. 3. Femur marrow from rat hypophysectomized for 2 months followed by daily injections of 0.01 mg thyroxin for 45 days (× 80). Note close resemblance to normal marrow above.

FIG. 4. Femur marrow from rat hypophysectomized for 2 months followed by daily injections of 0.05 mg thyroxin for 25 days (× 80). Note hyperplasia with loss of fat and increase in size of islands of erythroid elements.

FIG. 5. Femur marrow from rat hypophysectomized for 2 months followed by daily injections of 0.10 mg thyroxin for 10 days (× 80). Note normal appearance of marrow.

decrease in size and scattering of the islands of erythroid elements and an increase in fat. When slides were thoroughly mixed and treatments of animals unknown, a correct diagnosis of a non-treated hypophysectomized rat was made in all but one marrow. Best results were obtained with daily dose of 0.05 mg of thyroxin; the marrows in these animals were stimulated to a degree that islands of erythroid elements were larger than those found in normal controls and fat was decreased in amount when compared to normal. All rats were diagnosed as either normal or stimulated beyond normal. Treatment with 0.10 mg of thyroxin induced a marrow with normal appearance of erythroid elements and approximately a normal amount of fat. All but one of these marrows were diagnosed as normal. Treatment with the lowest dose, 0.01 mg, induced variable results. They were diagnosed as normal, containing too much fat, or questionable.

Discussion. Decreased oxygen need has been proposed as a cause for anemia which develops in hypophysectomized animals. This theory is supported by the fact that oxygen consumption is decreased after hypophysectomy, and that this low oxygen consumption is maintained even after the erythrocyte picture is returned to normal by transfusions(1). Therapeutic measures (except cobalt) proved effective in eliminating post-hypophysectomy anemia, have also elevated oxygen consumption; those found ineffective have not(2). This theory finds further support when one considers that the erythrocyte level after hypophysectomy does not continue to fall but plateaus at a fairly constant level 30 to 40 days after hypophysectomy. In addition, marrow in the hypophysectomized rat will respond normally under conditions of decreased oxygen tension(10), or if made more anemic by bleeding or by total body irradiation(11-14). Erythropoiesis does occur in hypophysectomized animals when the level of erythrocytes is lowered below the number actually needed by that animal.

In our experiment, injections of various doses of thyroxin into hypophysectomized rats induced increases in oxygen consumption which were accompanied in animals receiving the 2 highest doses by similar changes in total erythrocyte volume/100 g body weight. Increased erythropoiesis in bone marrows was observed in these groups. Similar results were reported(4) when cortisone and growth hormone were given in combination with the same doses of thyroxin. The 2 experiments differ with respect to response to lowest dose of thyroxin. Oxygen consumption was returned to normal in both instances, but the erythrocyte picture was normal only when thyroxin was combined with cortisone and growth hormone. However, it is apparent that when one increases the oxygen demand beyond this low-

est level the relationship between oxygen consumption and erythropoiesis can be demonstrated without cortisone and growth hormone. This correlation of oxygen consumption and erythropoiesis with thyroxin alone provides another instance in support of the oxygen need theory.

The marked loss of body fat in our experiment must be taken into consideration. There is certainly no doubt that body size must be considered when studying blood volumes as well as when determining oxygen consumption. It has been suggested that this loss of fat may not be similar to loss of a combination of all tissues and that the expression of total erythrocyte volumes/100 g body weight is not correct. The fact that these findings in total erythrocyte volumes/100 g body weight were accompanied by similar changes in marrow activity would seem to indicate that our conclusions are valid.

One cannot conclude from this experiment that post-hypophysectomy anemia is caused by a simple hypothyroidism in that the anemia of thyroidectomy is less severe than that of hypophysectomy. Loss of growth and adrenocorticotrophic hormones is also involved. Animals receiving our combined thyroxin-cortisone-growth hormone therapy are in much better health, body weight and growth are maintained and they are in better hematological condition than those receiving the same doses of thyroxin alone. What role these hormones play in the total process is unknown. It cannot be definitely determined whether growth hormone and cortisone act directly on the marrow or *via* some other mechanism. Evans *et al.*(15,16) have shown that there is a synergistic action of thyroxin and growth hormone on calorigenesis.

Summary. Hypophysectomized rats were treated with 0.01, 0.05 or 0.10 mg of thyroxin daily. Oxygen consumption in the 3 groups of animals was 100, 124 and 107% of normal; total erythrocyte volumes/100 g body weight were 83, 121 and 107% of normal. Hypophysectomized controls exhibited oxygen consumption 67% of normal with total erythrocyte volume/100 g body weight, 79% of normal. These data support the oxygen need theory of post-hypophysectomy anemia.

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