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To the Graduate Council:

I am submitting herewith a thesis written by Charles Major entitled "The Effect of Propylthiouracil and Thyroid Powder on the Restoration of Certain Components of the Liver After Partial Hepatectomy in the Rat." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Zoology.

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THE EFFECT OF PROPYLTHIOURACIL AND THYROID POWDER ON THE RESTORATION OF CERTAIN COMPONENTS OF THE LIVER AFTER PARTIAL HEPATECTOMY IN THE RAT

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Doctor of Philosophy

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by

Charles Major

December 1957

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INTRODUCTION

The process of regeneration after partial hepatectomy has been known since the work of Ponfick in 1890. After his original discovery of the process, an occasional pathologist made further studies, but in general little was done until, some forty years later, Higgins and Anderson (1931) reintroduced the process with a quantitative investigation of the restoration involved. This aroused a great deal of interest and, in the ensuing ten years, a number of cytological and anatomical studies were done. These confirmed the usefulness of the process and the immediate postwar period saw many quantitative biochemical investigations which mainly were with dietary influences on liver regeneration. The extraordinary amount of synthesis that occurs has made the process one of great intrinsic interest to those interested in problems of both normal and abnormal growth processes. It has further provided an outstanding tool for investigating the biosynthesis of compounds such as the nucleic acids which have extremely slow turnover rates in normal tissues.

There are many factors that have contributed to its utility. The operation is quite simple with negligible mortality. The tissue is easily accessible, relatively homogeneous, and, after the operation, is growing rapidly. It is an organ of great activity, of very diverse functions, and wide biochemical potentiality. It has been consistently noted that the left lateral and medial lobes constitute an almost fixed

fraction of the entire liver which, itself, has a relatively constant relationship to body weight. These constants permit work to be placed on a firm quantitative basis.

There exists a substantial body of literature to indicate that after partial hepatectomy and regeneration all liver components show a return to levels that are indistinguishable from the levels of normal liver. As a direct consequence the portion of the liver that is removed at operation provides a valuable reference point, giving both the original and the ultimate values for any item studied. The return to normal has been shown for such widely differing parameters as rate of glycolysis (Norriss, Blanchard, and Povolny, 1942), riboflavin content (Robertson and Kahler, 1942), and arginase and catalase activity (Greenstein, et al., 1942; Greenstein, 1945). The total number of cells remains slightly below normal values for some time. It is believed that the return of the functional mass depresses the stimulus to mitosis, whatever that stimulus may be so that it is not sufficient to induce the final slight increase required. The difference in number of cells is, however, quite small and by the fourteenth day of regeneration is quite difficult to demonstrate. In addition Harkness (1952 a) has presented data showing that collagen return is quite slow and takes more than six weeks.

The process of restoration proceeds in two distinct phases. The first of these lasts twenty-four hours and is one of cellular enlargement wherein nuclear and cytoplasmic volumes double while nucleolar

volume increases four-fold (Stowell, 1948). This is at the expense of extracellular and vascular space (Harkness, 1952 b). Throughout this period the mitotic rate is normal for the tissue but this value, one mitotic cell per ten thousand, is so low as to be inconsequential in view of subsequent events (Brues, Drury, and Brues, 1936). At the end of this twenty-four hour lag the second phase of regeneration begins with an eruption of mitoses among the parenchymal cells. Later in the second day, all other cell types have increased in the number of mitoses (Abercrombie and Harkness, 1951). At the peak of mitosis some thirty cells per thousand are in some stage of division. This peak has been variously placed by different investigators, as early as the twenty-fourth hour by Abercrombie and Harkness (1951) and as late as forty-eight hours by others (Marshak and Byron, 1945; Norriss, Blanchard, and Povolny, 1942). The consensus of numerous investigators is that the peak occurs during the first half of the second postoperative day.

The first twenty-four hours may well be termed the premitotic stage and actually constitutes the interphase for those cells which are about to divide. The time necessary for a liver cell to complete mitosis has been estimated to be forty-nine minutes (Brues, Drury, and Brues, 1936). Chromosomal complexity in regenerating liver shows no uncommon type of aberation and no evidence of polytene chromosomes such as occur in hepatic cancers (Biesele, 1944). Before entering mitosis one would expect the amount of desoxyribonucleic acid to double in each cell in accordance with the generally accepted theory of Swift

(1950) and Alfert (1950). Since mitosis only involves three per cent of the cells at its peak, one would expect a considerable dilution of this effect due to the number of inactive cells present. This is further complicated by the substantial polyploidy of the liver and its sizeable level of binucleate cells. The actual number of the binucleates declines during regeneration and they apparently participate in the mitosis. It is interesting that the number of these cells increases with increasing age of the animal and that consequently the regenerated liver shows a characteristic of "rejuvenation." All of these factors make it difficult to study the relationship of desoxyribonuleic acid to cell division but, despite this, Price and Laird (1950) and Thomson, et al. (1953) have presented data which do confirm the doubling of DNA per cell before division.

The period before extensive mitosis is one of cellular engorgement with lipoidal material, so much so that the liver becomes quite pale. Some authors have claimed that the tissue at this time is quite tough. This is contrary to the impression developed during the course of these experiments where it seemed that the liver, at this time, was extremely fragile and more difficult to handle. The factors of fat infiltration have been investigated by Sutherland (1956) and the lipid has been biochemically characterized by Ludewig, Minor, and Hortenstine (1939). The presence of the fat does not seem to impair the liver cells' capacity to proliferate.

In the course of regeneration there is extensive protein synthesis and this synthesis is not uniform, as witness the collagen, for example, which shows such a pronounced lag. The protein is the best indicator of regeneration as it steadily rises throughout the process and constitutes the bulk of the final dry weight. One should note that the initial rate of protein return is higher in animals fed a protein deficient diet. The final level is, of course, higher in animals fed adequate protein (Gurd, Vars, and Ravdin, 1948). This is an important consideration to be kept in mind when doing experimental work on the first few days of the restoration especially when the treatment given to animals has an effect on their appetite. The amount of ribonucleic acid present, which is closely related to protein synthesis in current thinking, rises to a peak at about forty-eight hours (Novikoff and Potter, 1948).

Information as to the influence of hormones on regeneration has been rapidly developed in the last few years but is still marked with contradictions. No single hormone has been shown to dominate the process, but the pituitary, adrenal cortex, and the thyroid may be involved.

Regeneration does proceed after hypophysectomy, but the amount regenerated is decreased according to many authors (Higgins and Ingle, 1939; Franceen, Brues, and Richards, 1938). This same result was even noted in toads (Taleisnik and Flores, 1948). None of these investigators are willing to attribute this decrease to a direct pituitary

effect preferring to note the profoundly disturbed metabolism and imply that any number of incidental factors may be responsible. The difficulties involved, for example, in the management of the anorexia are sizeable and often cited. Houssay (1948) says that there is no change attributable to the hypophysectomy. In contrast, Canzanelli, Guild, and Rapport (1949) say that dietary differences are not sufficient to account for the reduced restoration. Investigations not involving hepatectomy have shown that after both hypophysectomy and adrenalectomy, protein per cell is reduced and RNA levels either decrease or remain constant (Lee and Williams, 1952; Clark, 1953). This RNA drop is prevented by the administration of growth hormone (Reid, 1955). After partial hepatectomy subsequent to being hypophysectomized, animals have normal RNA values on the fourth day (Canzanelli, Guild, and Rapport, 1949). Reports of the effect of hypophysectomy on the DNA content of the liver cell have been contradictory even without the added complication of partial hepatectomy. Di Stephano, et al. (1952) reported that there was no effect while Bass, McArdle, and Grisham (1955) reported that DNA per nucleus is increased and that administration of growth hormone does not reduce it. Partial hepatectomy seems to have little effect on this component.

The influence of the adrenal cortex upon hepatic regeneration has been investigated by many groups. Canzannelli, Guild, and Rapport (1949) found no effect after adrenalectomy nor did Drabkin (1950). Ferrari and Harkness (1953) reported that adrenalectomized and control

animals did not essentially differ as to free amino acid content of the blood and liver at either six or twenty-four hours after partial hepatectomy. Friedgood, Vars, and Zerbe (1950) found that adrenalectomy decreased protein deposition as measured on the fourteenth day after partial hepatectomy. The decrease was eliminated by steroid therapy. In contrast Einhorn, Hirschberg, and Gellhorn (1954) showed that cortisone treatment increased fat deposition and lowered the liver's content of nucleic acids. It also reduced the cellularity of the tissue with the result that the nucleic acid content per cell was unchanged during regeneration. Mehrota and Mangalik (1955) found cortisone therapy decreased restoration. Tipton, Major, and Smothers (1956) have shown that adrenalectomy depressed the rate of restoration of enzyme activity and protein. Desoxycorticosterone acetate or adrenal cortical extract increased the rate of restoration of these components. In 1950 Arrigo and Pontremoli reported that ACTH increases the mass restoration.

Compared to the number of papers on other hormones, thyroid influences have not received a large amount of attention. Fogelman and Ivy (1948) found that thiouracil, a thyroid antagonist, increased mass restoration in animals killed on the eleventh post-operative day. The drug was given intraperitoneally (8 mg/100 gms body weight) daily beginning at the time of operation and continued to necropsy. Gershbein and Labow (1953) surveyed a large number of sulfur compounds using regenerating liver as the test process. The compounds included thiouracil

and thiourea. Thiouracil increased mass and thiourea decreased it. The procedure was that of Fogelman and Ivy (1948) except that the drugs were incorporated into the diet. In both of these studies the mortality was high and apparently no attempt was made to regulate the dietary intake. Silva (1950) found thiourea administration without effect on regeneration. Drabkin and Fetaco (1950) reported decreased restoration of mass after thyroidectomy. The most complete study of the thyroid in regeneration has been done by Canzanelli, Rapport, and Guild (1949) who noted that thyroidectomy decreased mass restoration which could then be restored to normal values by feeding thyroid powder. If thyroidectomized animals were given both thyroid powder and propyl-thiouracil, the restoration was returned to the level of animals merely thyroidectomized but there was a concommitant rise in RNA content of the liver. In these studies all animals were sacrificed on the fourth day. These changes could only be noted in very young rats. Older animals (four to six months of age) were completely refractory. Thyroid powder administration did give an increase in mass restoration and both thyroid powder and thiouracil gave a rise in the RNA concentration. While they do not comment on it, it appears that when both were given simultaneously the effect on the RNA concentration was additive. It was concluded that the thiopyrimidines inhibit by some process that is independent of an effect on the thyroid gland. An extremely pertinent experiment has been performed by Leatham and Seely (1947, 1948) who showed that thiouracil administered to normal and thyroidectomized

animals caused increased protein deposition in the livers of both groups. Many workers have noted that thiouracil increases the lipid content of the liver. Work in this laboratory (Tipton, Major, and Smothers, 1956) indicates that the restoration of total succinoxidase activity is depressed in animals on propylthiouracil as compared to thyroxine treated littermates. This was measured on the fourth day of regeneration at which time no distinction could be made on the basis of wet weight. In view of the many conflicting reports, it was felt that an investigation of the pre-mitotic and early mitotic stages under varying thyroid conditions would perhaps resolve some of the discrepancies, particularly in regard to rates of synthesis.

MATERIALS AND METHODS

Animals and Treatments

Male albino rats of the Sherman strain (Rockland Farms), 180-220 grams body weight, were used throughout these experiments. There were three experimental treatments so the animals were used in groups of three, each of which received one of the treatments.

Before being subjected to partial hepatectomy, all animals were force-fed the synthetic diet described in Table I. The force-feeding was done by means of a #8 French catheter tube attached to a 10 ml hypodermic syringe. In view of the thick consistency of the diet it was found necessary to cut off the tapered tip of the catheter to prevent frequent stoppages.

As is indicated in Table II, the animals received 10 ml of the synthetic diet split into two equal feedings on the fourth day before being operated upon, 16 ml in two doses on the third day preoperative, 20 ml similarly on the second day, and one 10 ml portion on the last day before the operation. This last was always given twenty-four hours before the operation was performed. After the operation, the animals received different amounts of the synthetic diet depending upon when they were scheduled for sacrifice. Those killed at fourteen hours after the operation were not fed at all. Those scheduled for sacrifice at twenty-four hours received 5 ml of the diet eighteen hours before death while those scheduled for the

forty-eight hour group received a 10 ml feeding at thirty-six hours and 5 ml at eighteen hours before sacrifice. All animals received 0.3 ml of the vitamin mixture (Table I), 0.3 ml of cod liver oil (Squibb), and 0.8 grams of salt mixture (#2, General Biochemicals, Inc.). All of these were given on the third day before the operation.

Of each group of three animals, one received 20 mgm per day of propylthiouracil, the second received 100 mgm daily of desiccated thyroid powder (Armour), while the third served as a control. The dosage of the propylthiouracil is equivalent roughly to 100 mgm per kgm body weight and the desiccated powder is equal to a level of 500 mgm per kgm body weight. These drugs were incorporated in the diet at the first feeding of each day. The propylthiouracil treatment was started one day before the dietary feeding and was given on that day suspended in five ml of water. After the operation only those destined for sacrifice at forty-eight hours were given drug doses and this as a single dose administered thirty-six hours before sacrifice.

Surgical Procedures and Liver Proportions

The procedure for partial hepatectomy was essentially that of Higgins and Anderson (1931). Animals were placed under ether anaesthesia and a midline incision of about 15 mm was made commencing at the xiphoid process of the sternum. The medial lobe was delivered through this, ligated at the peduncle with cotton thread, and excised. The left lateral lobe was then delivered, ligated, and removed in the same

manner. The incision was then closed with cotton thread. Mortality due to the operation was negligible. Most deaths were due to the hyperthyroid animals' sensitivity to the ether anaesthesia. In order to preclude this factor from disrupting the experimental groupings, it was customary to carry an extra thyroid-fed animal up to the operation to substitute if it proved necessary.

General Procedure for Analysis

The following procedure was carried out at operation and again at sacrifice. One gram of liver was quickly weighed on a Roller-Smith torsion balance and immediately homogenized in ice cold distilled water for one minute at 800 rpm in a Potter-Elvehjem homogenizor (Potter and Elvehjam, 1936) with a teflon pestle. The homogenate was then made up to a final volume of 10.0 ml with repeated washings of cold water. A 2.5 ml aliquot of this was then made up to 25.0 ml with cold water and this one per cent homogenate immediately frozen. Two 2.0 ml aliquots of the ten per cent homogenate were each delivered into tared centrifuge tubes containing 5.0 ml of cold ten per cent trichloroacetic acid. These were then fractionated in accordance with the procedure of Schneider (1945) modified in several ways. The precipitate remaining after the nucleic acid extraction was washed once with ninety-five per cent ethanol, the wash discarded and the residue dried, weighed, and considered to represent protein. It was then dissolved in the two per cent sodium hydroxide solution. The

procedure was further modified in that the lipid removal was done as three fifteen minute extractions with Bloor's alcoholic ether solution (3.1) at 60° C. It was felt that this was necessary in view of the heavy lipid infiltration occurring during hepatic regeneration.

The Schneider procedure gives four fractions: fraction one, the acid soluble phosphate; fraction two, the lipid phosphates; fraction three, the nucleic acids; and fraction four, the proteins. Fraction three was analyzed for ribonucleic acid by the orcinol reaction. Measurements were made at 660 millimicrons in 1 cm cells in a Beckman DU Spectrophotometer after color development for thirty minutes at 100° C. The standard employed was yeast RNA (California Foundation for Biochemical Research) and the computations based on the phosphorus content of the standard as described by Schneider (1945). The desoxyribonucleic acid of fraction three was computed similarly after determination by the Dische diphenylamine reaction measured at 595 millimicrons after color development for thirty minutes at 100° C. The standard employed was herring sperm DNA (California Foundation for Biochemical Research). In both the RNA and DNA determinations, blanks and standards were run each time. The RNA values were corrected for the DNA cross reaction with orcinol.

The phosphorus and nitrogen of each fraction, as well as that of the one per cent homogenate, was determined. The phosphorus method consisted of digestion with 10N H_2SO_4 containing a trace of selenium (2 mgm per liter) as suggested by Ma and Zuazaga (1942), and using the

supplemental digestion mixture of KHSO4 and Cu SO4 (Jones, Lee, and Peacocke, 1951). The subsequent color development was done by the method of Fiske and Subarrow (1925) with aminonaptholsulfonic acid (HARLECO) as the reducing agent. Standards and blanks were run each time and measurements made on a Klett-Summerson Photoelectric Colorimeter using a red filter. Nitrogen was determined by direct Nesslerization (Koch and McMeekin, 1924) following digestion with sulfuric acid (containing copper selenite, 20 mgm per liter) and accelerated by the addition of thirty per cent hydrogen peroxide (Merck's Superoxol or Baker's C. P.). Standards and blanks were run simultaneously and measurements made with the Klett-Summerson Photoelectric Colorimeter using the green filter (#50).

At operation and again at sacrifice a 500 mgm portion of the liver was taken, homogenized for thirty seconds (Bass and McArdle, 1956) in 3.0 ml of one per cent citric acid, washed with 1.0 ml with six per cent acetic acid containing crystal violet (80 mgm per liter). Aliquots were taken up in an inverted five ml volumetric pipette, shaken with a rotary motion, and delivered to a haemocytometer chamber. Nuclei were enumerated in twenty or thirty groups of sixteen small squares, depending on the homogeneity of the first twenty groups enumerated. It was found necessary to use a Petroff-Hauser Bacterial Counting Chamber in some cases (the fourteen hour postoperative group) where the fat present increased the optical density to the point where light would scarcely pass through the haemocytometer chamber.

Dry weight of the liver tissue was determined by drying the residual tissue overnight at 90° C. in tared weighing vials.

The left lateral and medial lobes constitute an almost constant fraction of the entire liver. To determine this fraction, fifteen male rats were sacrificed, the livers removed and weighed, and then reweighed with the left lateral and medial lobes detached. The animals used were of the same stock as those used for the regeneration studies.

Statistical Methods and Other Computations

The following formula was used to compute regeneration:

$$\frac{\text{Amount Present at Sacrifice - Amount Left in at Operation}}{\text{Amount Removed at Operation}} \times 100$$

An analysis of variance was run whenever the three groups were to be compared. The variance ratio "F" was determined and where it indicated a lack of homogeneity of the means, an analysis of these means was done by the sequential method of Duncan (1955). Where replication numbers differed, the modification of Kramer (1956) was used. The probability level for rejection of the null hypothesis was taken as 0.05 and termed significant, while those values below 0.01 are termed highly significant.

TABLE I

BASAL DIET

Sucrose	24 gm
Corn starch (Argo)	24 gm
Casein Vitamin-Free (General Biochemicals, Inc.)	16 gm
Wesson Oil	8 gm
Water	To 100 cc

100 ml of diet contains 318 calories.

VITAMIN MIXTURE[a]

Thiamine	2.0 mg
Riboflavin	4.0 mg
Pyridoxine	2.0 mg
Calcium Pantothenate	10.0 mg
Nicotinic Acid	50.0 mg
Biotin	0.02 mg
Inositol	100.0 mg
Choline	500.0 mg
Para-amino-benzoic Acid	100.0 mg
Distilled Water	enough to make 20 ml of mixture

[a]All vitamins are General Biochemicals, Inc., Chagrin Falls, Ohio.

TABLE II

VOLUME (ml) OF SYNTHETIC DIET
ADMINISTERED BEFORE OPERATION

Group	Time	Treatment[a]	Days Before Operation			
			4	3	2	1
I	A.M.	10 mg Propylthiouracil	5	8	10	10
	P.M.	None	5	8	10	--
II	A.M.	100 mg Desiccated Thyroid Powder	5	8	10	10
	P.M.	None	5	8	10	--
III	A.M.	None	5	8	10	10
	P.M.	None	5	8	10	--

[a]Group I received 10 mg propylthiouracil in 5 ml H_2O five days before operation.

RESULTS

The number of nuclei per gram wet weight of tissue is frequently termed the "cellularity" of the tissue. The values reported herein are extremely high compared to those of Price and Laird (1950) and others using their technique which employs a fifteen-minute homogenization in sucrose. According to Bass, McArdle, and Grisham (1956), this technique leads to excessive destruction of nuclei and they report that ten-second homogenization in either 0.85 per cent saline or citric acid gives values almost twice as high as those previously reported. An attempt was made to repeat the procedure of Bass, McArdle, and Grisham. This resulted in values that were higher than those of Price and Laird but lower than those published by the originators of the technique. It was noted that the amount of cellular debris present was excessive and often caused severe distortion of the sample distribution within the Petroff-Hauser Bacterial Counting Chamber. In order to eliminate this the homogenization time was increased to thirty seconds and a haemocytometer was used in place of the bacterial counting chamber. These changes did not affect the values obtained and reduced the variability within the counting chamber. Dr. Bass (personal communication) has recently obtained results lower than those previously reported and comparable to those reported here.

The diet which was force-fed the animals (Table I) was sufficient to maintain body weight after the second day of the regimen. This does

not guarantee that the animals absorb the same amount of nutrient from the alimentary tract but it does minimize this factor. All animals had appreciable mesenteric fat deposits at sacrifice. Force-feeding in this way may, however, constitute a stress and call forth some adrenal response.

The cellularity of each group was significantly different at operation. Those treated with thyroid powder had the greatest number of nuclei per gram of liver tissue, followed in order by the controls and those treated with propylthiouracil. The relative positions of the groups was unchanged throughout the process of regeneration. All values dropped significantly at fourteen hours and any treatment significance disappeared. The values are still significantly depressed at forty-eight hours except that at this time the group treated with thyroid powder showed a significant rise above the others but was still significantly lower than the value at operation. These values are tabulated in Table III. For all data reported the analysis of variance will be found in the Appendix and is labeled to correspond to the data table, thus Table III-A corresponds with Table III.

The desoxyribonucleic acid concentration per cell (Table IV) was the same for all groups at operation, fourteen hours after the operation, and forty-eight hours after the operation. All groups showed a rise at twenty-four hours, and this rise was a significant one for the thyroid treated group and for the control group.

When the data for RNAP concentration per cell (Table V) is subjected to the usual analysis of variance no distinction appears in the livers of the various groups at operation (Table V-A). This is due to the large variability within the experiment. Analyses made at different times used different homogenizers; other minor factors also varied from day to day. Since all experiments were carried out in groups of three individuals, containing an animal exposed to each treatment, a detailed analysis of variance was conducted (Table V-B) in an attempt to preserve the original matching and to decrease the effect of daily variations. When this is done the results of the treatments are significantly different at operation. The number of animals at each post-operative period was too small to permit the use of this statistical procedure at each post-operative period. At the time of operation the propylthiouracil-treated group had a higher RNAP concentration per cell than the thyroid-treated group which, in turn, had a higher concentration than the control group. After the operation all groups showed a rise in RNAP per cell. This rise was significant for the propylthiouracil group and the controls at fourteen hours. The rise became significant for the thyroid group at twenty-four hours. All groups showed declining levels at forty-eight hours which were not significant.

The propylthiouracil group showed no significant change in protein concentration per cell during the course of the experiment (Table VI). The mean value did rise but the difference was not

significant. In contrast the liver protein levels of both the control and hyperthyroid did show significant increases. This rise was significant for the thyroid fed group at twenty-four hours and for the control group at forty-eight hours. The protein content of the propylthiouracil group was significantly higher than the others at operation. The other two do not differ unless an analysis similar to that for the RNAP is done, in which case the three groups become discreetly significant in the order of decreasing values--propylthiouracil, controls, and thyroid treated (Table VI-B).

In all animals the ratio dry weight to wet weight (Table VII) was significantly higher at fourteen hours than at operation. The controls returned to normal at twenty-four hours while in the propylthiouracil group this return to normal was seen at forty-eight hours. The thyroid group did not return to normal within forty-eight hours although the values were declining.

The phorphorus of fraction one of the Schneider fractionation was derived from the soluble phosphorus compounds, predominantly inorganic phosphates, the phosphorylated sugars, and the nucleotides. No significant differences were found here in spite of a significant variance ratio (Table VIII). This can occur in analysis of means and methods have been evolved to resolve the components. These methods are not applicable here due to the limited number of means. By inspection one would presume that there was a slight drop in all groups after the operation with a chance that the thyroid treated group at

fourteen hours was lower than the others. There is some information which tends to confirm this. By error a diphenylamine test was made on a complete set of animals at operation and again at fourteen hours. This fraction contains those chromogens that interfere with the test, such as the hexose sugars which give a green color. It was noted that these chromogens were about equally present in all three groups at operation. At sacrifice all groups showed a sharp decline which was extremely pronounced in the hyperthyroid group. In the thyroid treated group they had, in fact, been almost completely eliminated.

The phosphorus of fraction two of the Schneider fractionation is essentially phospholipid phosphorus (Table IX). In all groups this rose to a peak at fourteen hours and declined below operative values at forty-eight hours. Only the terminal drop could be proven significant.

The preliminary survey of the relationship of the liver lobes to each other and to body weight indicated that the latter was more variable being extremely sensitive to the nutritional status of the animal. The ratio of liver weight to body weight showed a range of 0.031 to 0.050. The lowest values were found after starving animals for twenty-four hours. The range was 0.031 to 0.034. Animals fed normally gave a mean value of 0.039 while animals that were starved for twenty-four hours and then fed normally for the next twenty-four hours gave the high values (0.044 - 0.050). The relationship of the left lateral and medial lobes to the whole liver did not reflect this fluctuation and appeared to be age independent since the same value

was obtained for a sixty-seven gram rat as for a two hundred gram rat. These lobes comprised 71.0 $\pm$ 1.5 per cent of the entire liver mass. The amount of liver removed at operation was therefore multiplied by 1.41 to give the total wet weight of liver at operation.

The percentage restoration of the components is found in Figures 1, 2, and 3 and a more detailed presentation of the data is given in Table X. It should be noted that the events of the first fourteen hours were quite different from those of subsequent periods. The events of this period preceded cell division and there was a rapid rise in restoration of mass which is undoubtedly an expression of the fat infiltration which occurs. RNAP either did not decrease or rose slightly in this interval. All other components remained unchanged or showed a decrease which has appeared in other papers (Einhorn, Hirshberg, and Gellhorn, 1954).

After fourteen hours all components rose significantly and were analyzed at twenty-four and forty-eight hours. At these two time intervals certain relationships are evident. At twenty-four hours RNAP and mass were significantly higher than DNAP and protein. By forty-eight hours the situation had changed. RNAP was then significantly higher than mass and protein which, in turn, were significantly higher than DNAP restoration. Attention should be called to the fact that the interaction of hormone treatments and time verges on significance (Table X-A). Inspection of the data (Table X) shows that the greatest discrepancy of this type was found in the profoundly

reduced protein restoration of the propylthiouracil treated group at forty-eight hours. It is realized that statements made about such a possible interaction are subject to serious statistical question.

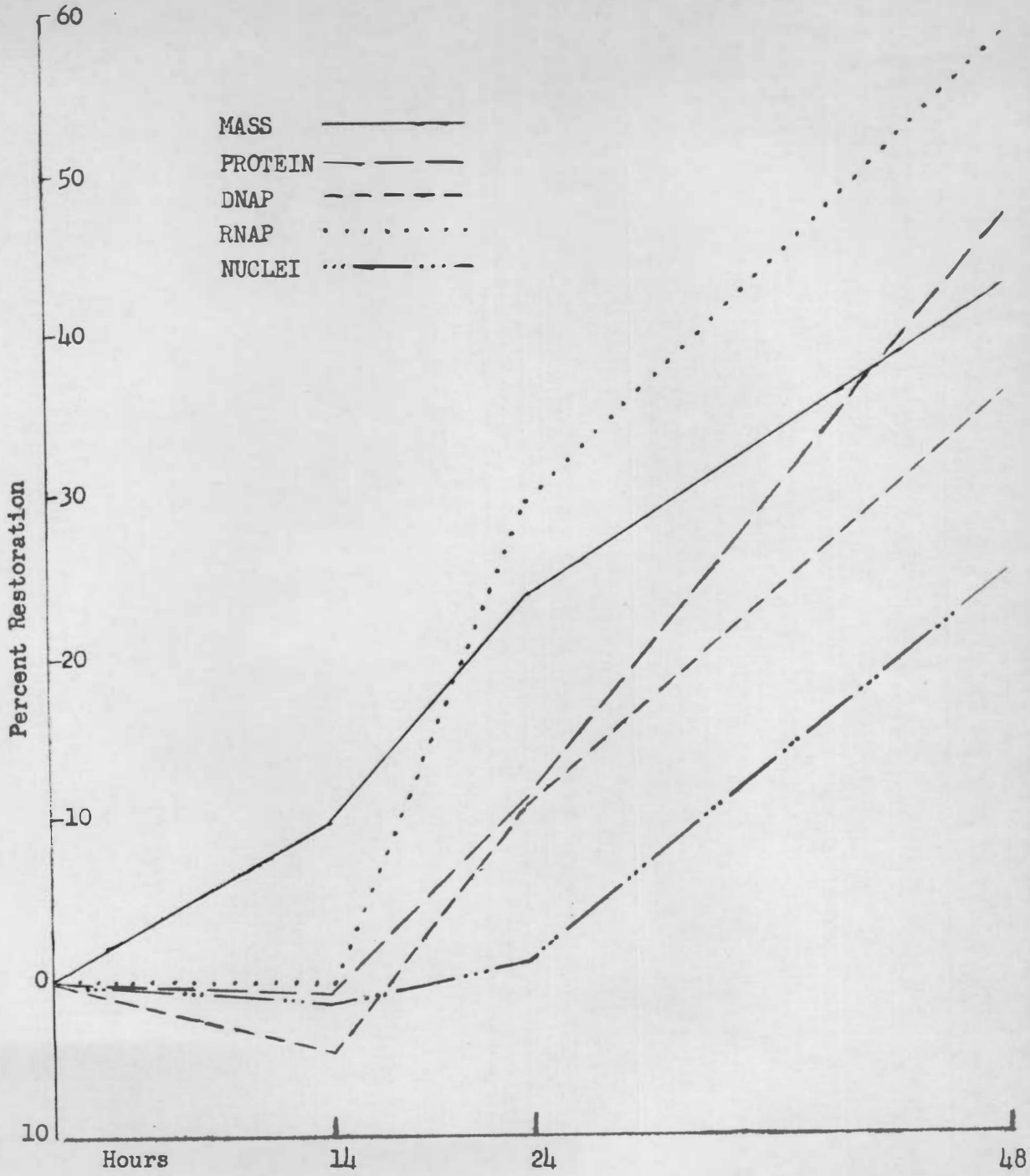


Figure 1. Percentage restoration--thyroid powder treated group

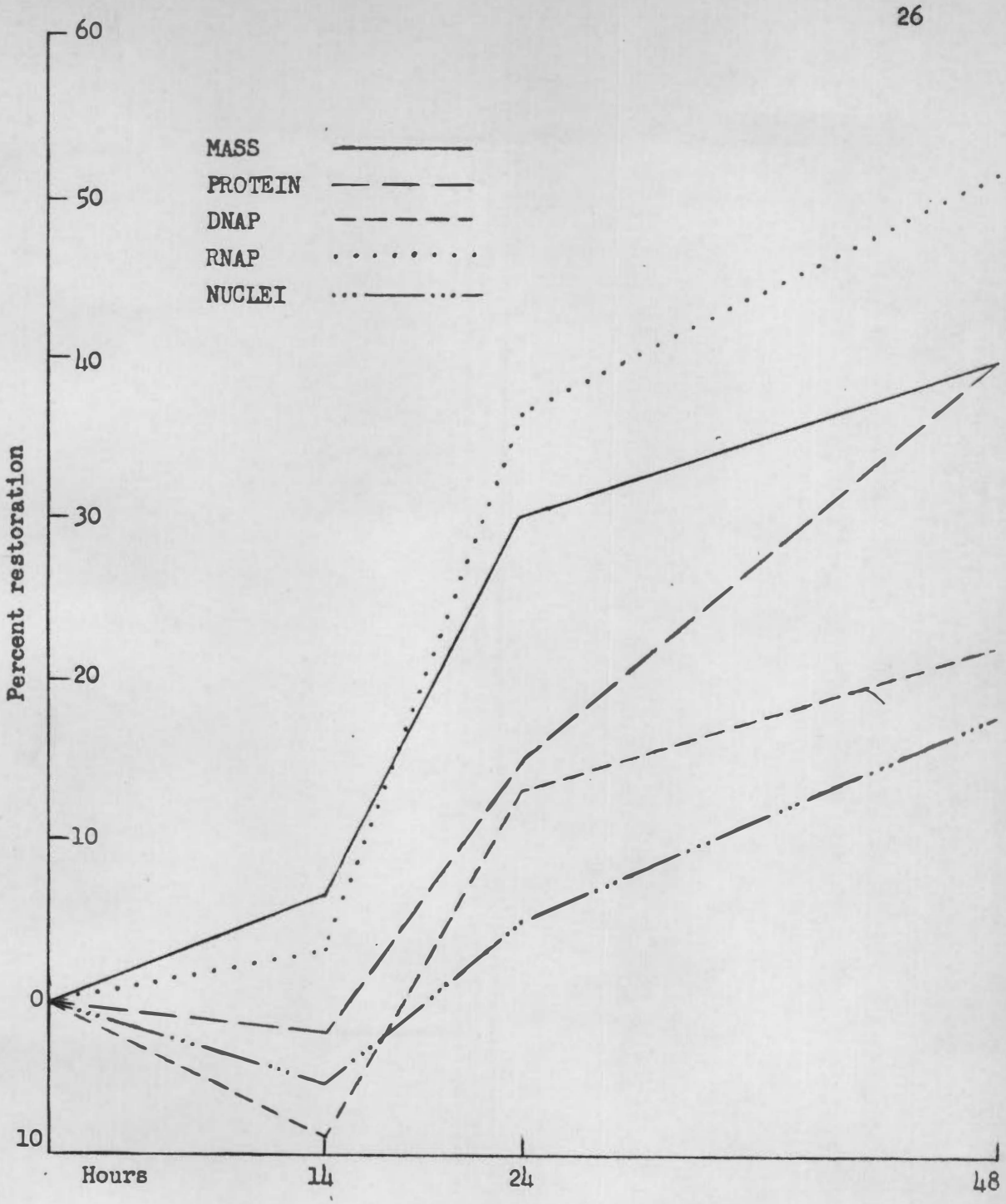


Figure 2. Percentage restoration--control group

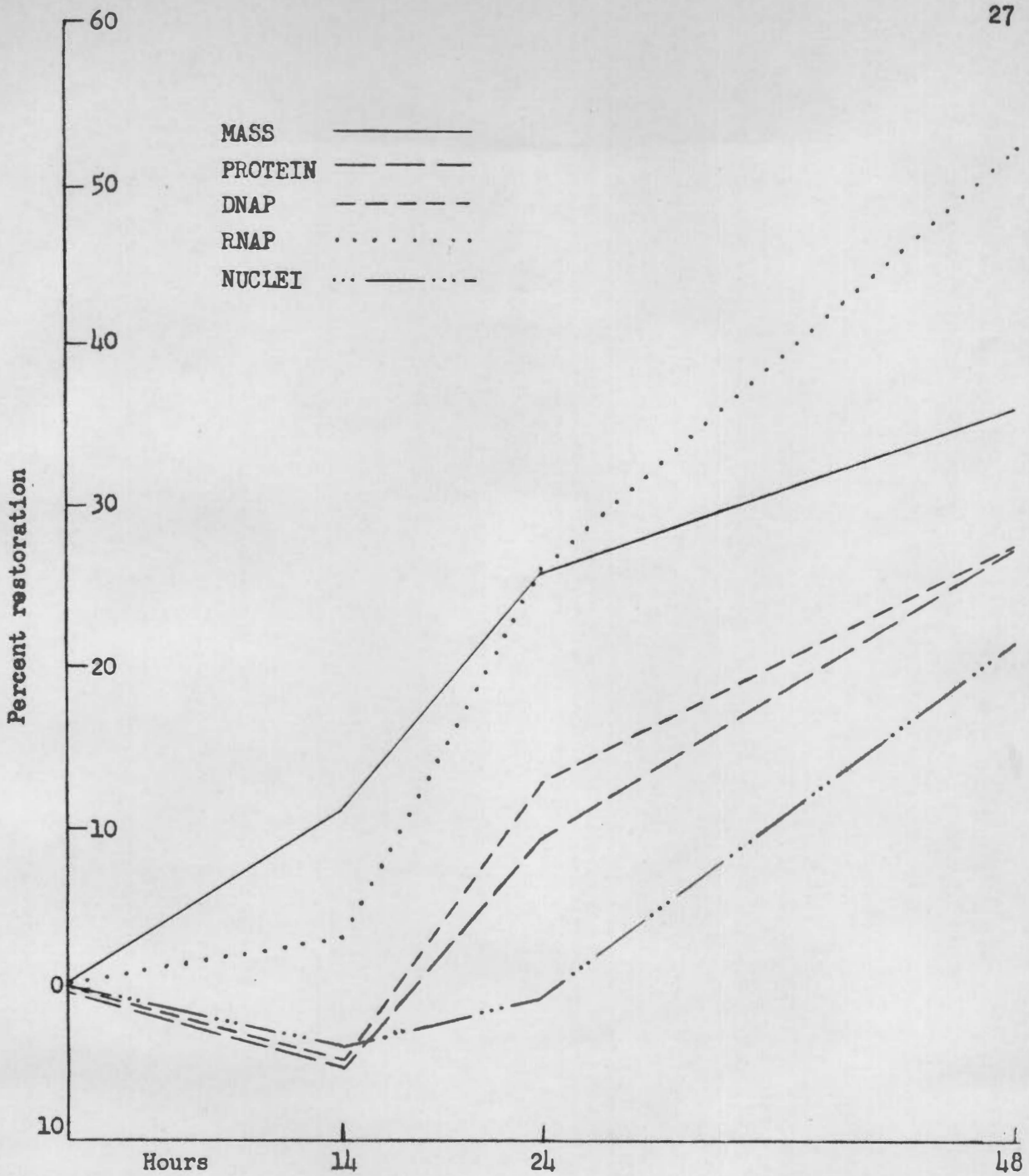


Figure 3. Percentage restoration--propylthiouracil treated group

TABLE III

NUCLEI COUNTS

(Nuclei x 10^8 per gram wet weight of tissue)

Treatment	At Operation		Hours After Operation 14	24	48
Propylthiouracil	2.67 (9)	**	1.95 (2)	1.71 (3)	2.02 (3)
	*				*
Desiccated thyroid powder	3.18 (9)	**	2.13 (2)	1.97 (3)	2.57 (3)
	*				*
Controls	2.95 (9)	**	2.00 (2)	1.91 (3)	2.21 (3)

Number of animals indicated within parentheses.

*Indicates $P < .05$ for the values between which it is located.

**Indicates $P < .01$ for the values between which it is located.

TABLE IV

DESOXYRIBONUCLEIC ACID PHOSPHORUS

(Grams x 10^{-12} per cell)

Treatment	At Operation	Hours After Operation		
		14	24	48
Propylthiouracil	1.28 (9)	1.30 (3)	1.59 (3)	1.22 (3)
Desiccated thyroid powder	1.25 (9)	1.29 (2) *	1.65 (3) *	1.23 (3)
Controls	1.33 (9)	1.27 (3) *	1.62 (3) *	1.19 (3)

Number of animals indicated within parentheses.

*Indicates $P < .05$ for the values between which it is located.

TABLE V

RIBONUCLEIC ACID PHOSPHORUS

(Grams x 10^{-12} per cell)

Treatment	At Operation	Hours After Operation		
		14	24	48
Propylthiouracil	2.883 (9) *	3.369 (3) **	4.439 (3)	4.180 (3)
Desiccated thyroid powder	2.681 (9)	2.961 (2) **	4.467 (3)	4.159 (3)
Controls	2.473 (9) *	3.025 (3) **	4.159 (3)	3.944 (3)

Number of animals indicated within parentheses.

*Indicates $P < .05$ for the values between which it is located.

**Indicates $P < .01$ for the values between which it is located.

TABLE VI

PROTEIN

(Grams x 10^{-12} per cell)

Treatment	At Operation	Hours After Operation 14	24	48
Propylthiouracil	737 (18) *	706 (6)	821 (6)	835 (6)
Desiccated thyroid powder	586 (18)	587 (4) *	723 (6)	790 (6)
Controls	646 (18)	671 (5)	764 (6) *	892 (6)

Number of animals indicated within parentheses.

*Indicates $P < .05$ for the values between which it is located.

TABLE VII

PERCENTAGE DRY WEIGHT

Treatment	At Operation	Hours After Operation		
		14	24	48
Propylthiouracil	27.39 (9) *	31.72 (3)	30.70 (3) *	27.68 (3)
Desiccated thyroid powder	25.94 (9) *	32.96 (2)	30.38 (2)	29.84 (2)
Controls	28.13 (9) *	34.25 (3) *	30.13 (2)	29.02 (3)

Number of animals indicated within parentheses.

*Indicates $P < .05$ for the values between which it is located.

TABLE VIII

ACID SOLUBLE PHOSPHORUS

(Grams x 10^{-6} per gram wet weight of tissue)

Treatment	At Operation	Hours After Operation		
		14	24	48
Propylthiouracil	1032 (9)	937 (3)	949 (3)	866 (3)
Desiccated thyroid powder	916 (9)	827 (2)	936 (3)	872 (3)
Controls	973 (9)	894 (3)	917 (3)	859 (3)

TABLE IX

PHOSPHOLIPID PHOSPHORUS

(Milligrams per gram wet weight tissue)

Treatment	At Operation	Hours After Operation		
		14	24	48
Propylthiouracil	1369 (8)	1565 (3)	1487 (3) *	960 (2)
Desiccated thyroid powder	1326 (8)	1441 (2)	1177 (3)	963 (2)
Controls	1306 (8)	1485 (3)	1319 (3) *	893 (2)

Number of animals indicated within parentheses.

*Indicates $P < .05$ for the values between which it is located.

TABLE X

PERCENTAGE RESTORATION AT TWENTY-FOUR AND FORTY-EIGHT HOURS

Component	Treatment	Twenty-four Hours	Forty-eight Hours
RNAP	Propylthiouracil	25.8	51.9
	Desiccated thyroid	29.8	58.4
	Controls	36.5	51.5
DNAP	Propylthiouracil	12.3	27.4
	Desiccated thyroid	11.0	36.2
	Controls	12.8	22.0
PROTEIN	Propylthiouracil	8.7	27.0
	Desiccated thyroid	11.5	47.5
	Controls	14.6	39.9
MASS	Propylthiouracil	25.6	35.7
	Desiccated thyroid	24.0	43.0
	Controls	30.0	39.9

Each cell contains the mean of three replications.

All forty-eight hour values are significantly higher than corresponding twenty-four hour values.

At twenty-four hours RNA and MASS constitute a single population.

At forty-eight hours PROTEIN and MASS constitute a single population of lower mean ($P < .01$) than RNAP and of higher mean ($P < .05$) than DNAP.

DISCUSSION

Let us consider the course of regeneration during the first two days. This can be seen quite clearly in Figures 1, 2, and 3 regardless of the treatments. All constituents of the liver showed increases after variable lag periods. In these graphs there is only one component, protein, that actually contributes any substantial portion to the total mass. All other compounds displayed are present in almost negligible amounts and can be disregarded as components of wet weight. Immediately after the operation the liver mass began to increase. This increase was not due to the mere accumulation of water as the ratio, dry weight to wet weight, was increasing at this time (Table VII). Protein levels had not yet risen and the experiment was deliberately designed to keep carbohydrate levels minimal although this is scarcely necessary since carbohydrate concentrations decline markedly during regeneration (Gurd, Vars, and Ravdin, 1948). The difference between the two curves, mass and protein restoration, must then represent the lipid infiltration of the tissue. By noting the relationship between the two curves one can follow the general course of events. When the protein curve crosses the mass curve it indicates that protein synthesis is not proceeding rapidly enough to compensate for the rapid drop in lipid present. As lipid disappears the two curves should coincide. Divergence of the two curves would indicate that greater increments of fat are being deposited relative to protein. If the lines remain parallel it would indicate a persistence of the

lipid in the tissue.

During the first fourteen hours these curves diverged and the tissue was flooded with lipoidal material. The reason for this has not been established. Perhaps the liver enzyme systems always function close to their capacity for handling lipids. If this were so then any damage to the liver that reduces the amount of functional tissue present would cause lipids to pile up in the liver cells. Fatty infiltration of the liver is a classic symptom of hepatic malfunction. The rising lipid content of the liver may be the stimulus for the ensuing mitosis. It would provide a fine feedback mechanism of decreasing intensity with increasing hepatic tissue. One hesitates to adopt this since this lipid increase consists predominantly of neutral fatty acids (Ludewig, Minor, and Hortenstine, 1939) and these compounds are not highly reactive and their turnover is rather slow. It should further be noted that a high fat diet is distinctly unfavorable to regeneration if continued past the operation (Sutherland, 1956; Moise and Smith, 1924).

The second feature of regeneration that was immediately noted was the early rapid rise of ribonucleic acid. This constituent is a product of synthesis within the cell and its rise cannot be accounted for on the basis of external depots and overwhelmed liver enzymes. It was therefore the first detectable change in the cellular processes that heralded the subsequent restoration. The return of this component preceded all others throughout the first two days. Price and Laird

(1950) believe that it doubles in concentration in the combined microsomal-supernatant fraction before the onset of mitosis in the liver. The number of cells in mitosis was not determined in these experiments so definite statements on this point cannot be made. If we approach this question using slightly different parameters we can add something. It is almost certain that little mitosis occurred before twenty-four hours. The increase in desoxyribonucleic acid concentration at this time is a good indicator of the number of cells that are about to divide. The DNA was elevated some twenty-six per cent at this time, while the RNA was increased about sixty-three per cent. Since DNA doubles before mitosis, the RNA change was then far more than a mere doubling. It has been shown that this increase is predominantly cytoplasmic (Price and Laird, 1950; Stowell, 1948; Rapport, Canzanelli, and Guild, 1948). The RNA is probably involved in changes of the microsomal units. If these were to be increased rapidly they might develop a lead over cell division and be better able to maintain normal or near normal small particle populations in the face of the intense mitosis that is about to occur.

Desoxyribonucleic acid concentration remained unchanged for the first fourteen hours, rose then to a marked peak at twenty-four hours, and by forty-eight hours had returned to normal. DNA and cell number returned at about the same rate but the DNA rise preceded the cell number increase by several hours. This is as one would expect. Chromosomal doubling occurred followed by mitosis and normal values

reappeared.

The process of protein restoration had a lag period of no more than fourteen hours and proceeded at a rate only exceeded by that of ribonucleic acid. Numerous investigators have proposed that RNA is, in some way, the organizer of protein synthesis. Experimental evidence for this is now accumulating and the article by Borsook (1955) should be consulted for an extensive summary of current trends in this field. Jeener (1953) goes so far as to say, "the rate of increase of cellular proteins is proportional to the quantity of RNA which is in excess of a basal quantity concerned with the renewal of the existing protein." The data of this experiment are consistent with such an explanation. During the second day lipid levels decreased more rapidly than protein levels rose which may be seen from the convergence of these curves. Since one may, in a broad sense, equate "functional" tissue with "enzymatic" protein of the tissue, this is not unexpected. If the lipid removal is enzymatic one would not expect the lipid decrease to be a linear function of protein present. Further confirmation that this removal is enzymatic can be seen in the fact that Coenzyme A, which is important in lipid metabolism, reduces the extent of the infiltration. No investigation of the role of lipid enzymes in regeneration has been made to date. Enzymes are, of course, essentially protein and some studies have been done on this point. The result seems to depend on the enzyme for some investigated show abnormally high activities during regeneration while others remain the same or even

drop below normal (Novikoff and Potter, 1948; Oppenheimer and Flock, 1947; Rosenthal, Fahl, and Vars, 1952). The lipid present may dilute the activity of an enzyme and the use of wet or dry weight for the expression of enzymatic activity is misleading and should be avoided. The lipid apparently does not interfere with the functioning of succinoxidase nor cytochrome oxidase. That these did not decrease and possibly increased at twenty-four hours after operation was noted in a survey experiment by the author. This was only so if the basis "fat-free dry weight" was used to express the results. It should be noted that during the first two days protein was being synthesized more rapidly than the cells were dividing and thus the amount of protein per cell was increasing.

Let us now turn to the hormonal effects upon the process of regeneration. It must be borne in mind that the liver is the principle organ whereby circulating thyroid hormone is eliminated (Kellaway, Hoff, and Leblond, 1945). The removal of a large part of this organ may affect the routine maintenance of the levels of this hormone and cause an increase in the amount present in the circulation. Whether this is sufficient to be of importance physiologically has not yet been proven. If it were true we would again have a feedback situation with an obvious mechanism of stimulation and regulation. However, this effect has never been investigated and its very existence remains a matter of conjecture.

At the time of operation the three groups already differed as to certain factors. The cellularity of each differed, there being a larger number of nuclei per gram wet weight in the hyperthyroid group with successively lower numbers in the controls and propylthiouracil treated groups. Protein concentration per cell showed the same gradient inverted, there being a lower concentration per cell in the thyroid fed group than in the normal, which, in turn, is lower than the propylthiouracil group. The RNA per cell had a different pattern with both drug treatments being higher than the controls. Of the two higher groups, the propylthiouracil group was the highest. There is apparently some difference in the response of cellularity and protein on the one hand, and RNA on the other. If this were a simple one factor experiment with only linear relationships one would expect a constant relationship to show up between the hyperactive, normal, and hypoactive groups since the experiment would then be the equivalent of a single drug treatment at three levels of concentration. Apparently the situation is not that simple.

During the course of regeneration the order shown at operation was maintained for cellularity, all groups showing an initial drop and recovery taking place. The recovery was, however, best for the thyroid treated group. The thyroid treated and the control group both showed rising protein per cell values, while the propylthiouracil treated group did not show this. In consequence the order of these changed from the order at operation. As to the whole organ, the propylthiouracil

group (Figure 3) showed a difference of pattern with an apparent persistence of the lipid as evidenced by the failure of the mass and protein curves to converge. The DNA rise at twenty-four hours was significant only for the control and thyroid treated groups. The propylthiouracil treated group was, however, almost elevated significantly. Thus we see two related pieces of information, low cellularity and low DNA rise, which would indicate that the mitotic rate is lower in the propylthiouracil treated group than in the others. To clarify this point it would be necessary to use shorter time intervals to resolve these portions of the curves. It is possible that we are comparing an already declining point on the hyperthyroid curve with the absolute peak of the hypothyroid curve.

The total restoration of RNA was the same for all groups at fourteen, twenty-four, and forty-eight hours after the operation. On a per cell basis there was no significant difference between the treatments but again we get the peculiar grouping of the hyper and hypo being above the mean value of the controls.

We can approach an evaluation of these patterns in two ways. The first method would assume that the simplest possible situation exists here. It is a matter only of an active gland, a normal level, and a blocked gland. In this model we assume that all effects are due exclusively to thyroid hormone or lack of it. Cellularity would then be directly proportionate to the amount of this hormone in circulation and the effect might be on DNA synthesis. Protein per

cell would vary inversely with the hormone's concentration. The hormone would then cause a decrease in protein synthesis or an increase in protein degradation. The effect could also be due to an increase in the protein turnover rate. All the evidence in hyperthyroidism indicates heightened tissue activity and elevated basal metabolism. At the cellular level this should mean increased enzymatic activity which could occur with an increase in amount of enzyme protein or with an increase in the activity of an unchanged amount of enzyme protein. It could, however, mean a faster turnover of protein with subsequently increased cellular adaptivity to changing substrata levels. Since the hyperthyroid animals had an increased hepatic cellularity without a change in DNA per cell, mitosis must have been stimulated. The elevated RNA per cell in the treated groups calls for an explanation based on a minimal effect at the control level with increased production on either side of this point. However, where RNA increases one looks for increased protein production and increased protein per cell, yet the two treated groups have differing protein responses.

This pattern may be approached in a second way. It is difficult to envisage any effect of desiccated thyroid powder that would not primarily be an effect of its active iodinated component. In contrast, propylthiouracil is an analog of uracil, a major component of ribonucleic acid, and this is the constituent which is behaving paradoxically. In this second approach let us assume that propylthiouracil has an effect that is independent of its thyroid blocking effect. This

would not change the response of the thyroid fed and control groups which would then act as postulated in the simplest approach already discussed. In the propylthiouracil group the situation would be more complex. The propylthiouracil molecule would enter the RNA molecule of the tissues and interfere with the normal behavior of this compound, possibly blocking a certain amount of its activity, and possibly interfering with its turnover rate at the same time. The block would not be complete and some protein would be laid down but at a slower rate. An interference with mitosis need not be postulated as an independent effect but it might occur since both uridine and uridylic acid are incorporated into both the RNA and DNA pyrimidines (Chargoff and Davidson, 1956). It was not believed that the free pyrimidine was so incorporated, but a recent paper by Welch (1957) reports that it is. The incorporation into DNA would probably be as thymine which is a methylated uracil compound. Use of labeled propylthiouracil should easily establish whether it is in fact incorporated into the RNA of the liver.

There are many scattered reports in the literature which would lend credence to this proposed mode of action. This evidence, however, is for the compounds methylthiouracil or thiouracil itself. The propyl analog is far more active than these in thyroid gland suppression. It is a more recent development and, except in thyroid therapy, has not been extensively investigated. It has been shown that thiouracil inhibits Tobacco Mosaic Virus production (Commoner and

Mercer, 1952) and that this is due to its incorporation into the RNA (Jeener and Rosseels, 1953). The effect can be reversed by uracil. Thiouracil reduces the germination of cress seeds (Trotter, 1949). The effect is competitively reversed by uracil. Thiouracil inhibits the growth of many microorganisms, Escherica coli (Strandskov and Wyss, 1952), tubercle bacillus (Duca and Steinback, 1946), and Lactobacillus casii (Wolf, 1948). In each case this growth inhibition is reversed by uracil. Thiouracil also inhibits seedling growth (Ber, 1949), and is reversed by uracil. There have been two papers on the nucleic acid of the thyroid gland, but the results conflict badly. Rerebek and Rerebek (1947) found thiouracil decreased both RNA and DNA of the gland and caused an increased metaphase period of glandular cells in mitosis. In contrast Suzuki (1953) found that methyl thiouracil induced a doubling of DNA and tripling of RNA in the gland.

All evidence at present points to the thiouracil action on the gland as an action that blocks organic binding of iodine, possibly through interference with the enzyme tyrosinase and possibly through interference with peroxidoses. No final proof has, as yet, been produced for any of these as the mechanism of antithyroid activity and there is available considerable evidence that the binding of iodine occurs within protein rather than from the free amino acids present (Glandular Physiology and Therapy, 1954). In animals treated with thyrotropin and antithyroid drugs there appear new proteins which differ from those produced by the normal gland (Derrien, et al., 1949).

While the action of propylthiouracil on the thyroid gland need not be the same as its effect on the liver cell, it would certainly be a tidier picture and the evidence as yet does not preclude this possibility.

Barker, Kiely, and Lipner (1949) commented, on the basis of studies on the response of thyroidectomized and thiouracil treated animals to thyroxine, that the effect of thiouracil "seemed to extend beyond the gland." If thiouracil has an effect independent of its gland blocking action, then one would expect the rise of RNA during partial hepatectomy to occur even in thyroidectomized animals. One experiment of this type has been done by Canzanelli, Guild, and Rapport (1949) who noted that on the fourth day of regeneration, very young animals treated in this way had an elevated RNA content. This did not appear in older animals.

More complicated explanations of the effect of these treatments on regeneration can be devised involving the pituitary. The hypothesis offered is, however, one that lends itself to further investigation quite readily, and should be pursued further before additional complications are assumed.

SUMMARY

The effect of the administration of thyroid powder and propylthiouracil in a forced feeding regimen upon the restoration of certain components of the rat liver has been studied at fourteen, twenty-four, and forty-eight hours after partial hepatectomy in the rat. Cellularity was determined and found to be highest in the thyroid group, intermediate in the controls, and lowest in the propylthiouracil group, both at operation, and throughout the subsequent regeneration. All groups declined in cellularity after the operation with only the thyroid group showing a significant return towards normal by the forty-eighth hour.

After the operation RNAP per cell, protein per cell, and total mass all rose significantly in the thyroid and control groups. The propylthiouracil group showed a significant increase in RNAP and total mass. In all groups DNAP per cell rose at twenty-four hours and had returned to normal values by forty-eight hours. This rise was significant only for the thyroid and control groups. The total restoration in the organ as a whole indicated that lipid infiltration was more persistent in the propylthiouracil group which also seemed to restore protein at a lower rate than did the other groups.

BIBLIOGRAPHY

BIBLIOGRAPHY

Abercrombie, M., and Harkness, R. D., 1951. "The Growth of Cell Populations and the Properties in Tissue Culture of Regenerating Liver of the Rat," Proc. Roy. Soc. B., 138:544-561.

Alfert, M., 1950. "A Cytochemical Study of Oogenesis and Cleavage in the Mouse," Jour. Cell. and Comp. Physiol., 36:381.

Arrigo, L., and Pontremol, S., 1950. "Action of ACTH on Regenerating Liver After Partial Hepatectomy," Boll. Soc. Ital. Biol., 26 (3): 258-259.

Barker, S. B., Kiely, C. E. Jr., and Lipner, H. J., 1949. "Metabolic Effects of Thyroxine Injected into Normal, Thiouracil-treated, and Thyroidectomized Rats," Endocrinology, 45:624-627.

Bass, A. D., McArdle, A. H., and Grisham, J. W., 1955. "Effect of Hypophysectomy on Liver Nucleoproteins," Proc. Soc. Exp. Biol. and Med., 89:7-9.

_____, 1956. "Nuclei Counts on Rat Liver Homogenates," Science, 123:372.

Ber, v. A., 1949. "Auxine und Nucleinsauren," Experientia, 5:455-460.

Biesele, J. J., 1944. "Chromosome Complexity in Regenerating Liver," Cancer Research, 4:232-235.

Boorsook, H., 1956. "The Biosynthesis of Peptides and Proteins," Jour. Cell. and Comp. Physiol., 47 (Supplement):35-80.

Brues, A. M., Drury, D. R., and Brues, M., 1936. "A Quantative Study of Cell Growth in Regenerating Liver," Arch. Path., 22:658-763.

Canzanelli, A., Guild, R., and Rapport, D., 1949. "Pituitary and Adreno-cortico Relationships to Liver Regeneration and Nucleic Acids," Endocrinology, 45:91-95.

Canzanelli, A., Rapport, D., and Guild, R., 1949. "Control of Liver Regeneration and Nucleic Acid Content by the Thyroid with Observations on the Effect of Pyrimidines," Amer. Jour. Physiol., 157:225-233.

Chargoff, E., and Davidson, J. N., 1955. The Nucleic Acids, New York, N. Y. Achademic Press Inc.

Clark, I., 1953. "The Effect of Cortisone upon Protein Synthesis," Jour. Biol. Chem., 200:69-76.

Commoner, B., and Mercer, J., 1952. "The Effect of Thiouracil on the Rate of Tobacco Mosaic Virus Biosynthesis," Arch. Biochem. and Biophys., 35:278.

Derrien, Y., Michel, R., Pedersen, K. O., and Roche, J., 1949. "Recherches sur la Preparation et sur les Proprietes de la Thyroglobuline Pure," Biochem. et Biophys. Acta., 3:436.

Di Stefano, H. S., Bass, A. D., Diermeier, H. F., and Tepperman, J., 1952. "Nucleic Acid Patterns in Rat Liver Following Hypophysectomy and Growth Hormone Administration," Endocrinology, 51:386-393.

Drabkin, D. L., 1950. "Cytochrome c Metabolism and Liver Regeneration--The Influence of Adrenalectomy," Jour. Biol. Chem., 182:351-357.

Drabkin, D. L., and Fetaco, G. M., 1950. "Cytochrome c Metabolism and Liver Regeneration--The Influence of Thyroid Gland and Thyroxine," Jour. Biol. Chem., 182:335-349.

Duca, C. J., and Steinback, M. M., 1946. "The Effect of Thiouracil upon in vitro and in vivo Growth of the Tubercle Bacillus," Amer. Rev. Tuberc., 53:594.

Duncan, D. B., 1955. "Multiple Range and Multiple F Tests," Biometrics, 11:1-42.

Einhorn, S. L., Hirschberg, E., and Gellhorn, A., 1954. "Effects of Cortisone on Regenerating Rat Liver," Jour. Gen. Physiol., 37:559-574.

Ferrari, V., and Harkness, R. D., 1954. "Free Amino Acids in Liver and Blood After Partial Hepatectomy in Normal and Adrenalectomized Rats," Jour. Physiol., 124:443-63.

Fiske, C. H., and Subarrow, Y., 1925. "The Colorimetric Determination of Phosphorus," Jour. Biol. Chem., 66:375-400.

Fogelman, M. J., and Ivy, A. C., 1948. "Effect of Thiouracil on Liver Regeneration," Amer. Jour. Physiol., 153:397-401.

Franceen, C. C., Brues, A. M., and Richards, R. L., 1938. "The Effect of Hypophysectomy on the Restoration of the Liver Following Partial Hepatectomy in Rats," Endocrinology, 23:292-301.

Friedgood, C. E., Vars, H. M., and Zerbe, J. W., 1950. "Role of Adrenal Cortex in Liver Regeneration," Amer. Jour. Physiol., 163: 354-363.

Gershbein, L. L., and Labow, J. A., 1953. "Effect of Various Sulfer Compounds on Rat Liver Regeneration," Amer. Jour. Physiol., 173:55-57.

Glandular Physiolgy and Therapy (5th Ed.), 1954. Philadelphia, Pa. J. P. Lippincott Company.

Greenstein, J. P., 1945. Report of AAAS Research Conference on Cancer, Washington, p. 192.

Greenstein, J. P., Edwards, J. E., Andervont, H. B., and White, J., 1942. "Comparative Enzymatic Activity of Transplanted Hepatomas and of Normal, Regenerating, and Fetal Liver," Jour. Nat. Cancer Inst., 3 (1):7-17.

Gurd, F. N., Vars, H. M., and Ravdin, I. S., 1948. "Composition of the Regenerating Liver After Partial Hepatectomy in Normal and Protein Depleted Rats," Amer. Jour. Physiol., 152:11-21.

Harkness, R. D., 1952 a. "Collagen in Regenerating Liver of the Rat," Jour. Physiol., 117:257-266.

_____, 1952 b. "Changes in the Liver of the Rat After Partial Hepatectomy," Jour. Physiol., 117:267-277.

Higgins, G. M., and Anderson, W., 1931. "Restoration of the Liver of the White Rat Following Partial Surgical Removal," Arch. Path., 12:186-202.

Higgins, G. M., and Ingle, D. J., 1939. "Regeneration of Liver in Hypophysectomized White Rats," Anat. Rec., 73:95-104.

Houssay, H. E. J., 1948. "Regeneracion del Higado en el Sapa Hipopisoprivo," Rev. Soc. Argen. Biol., 24:174-176.

Jeener, R., 1953. "Ribonucleic Acid and Protein Synthesis in Continuous Cultures of Polytomella caeco," Arch. Biochem. and Biophys., 43:381.

Jeener, R., and Rosseels, J., 1953. "Incorporation of 2-Thiouracil 35_S in the Ribose Nucleic Acid of Tobacco Mosaic Virus," Biochem. et Biophys. Acta, 11:438.

Jones, A. S., Lee, W. A., and Peacocke, A. R., 1951. "The Determination of Phosphorus in Desoxypentose Nucleic Acids," Jour. Chem. Soc., p. 623.

Kellaway, P. E., Hoff, H. E., and Leblond, C. P., 1945. "The Response to Thyroxine After Subtotal Hepatectomy," Endocrinology, 36:272-279.

Koch, F. C., and McMeekin, T. L., 1924. "A New Direct Nesslerization Micro-Kieldahl Method and a Modification of the Nesslor-Folin Reagent for Ammonia," Jour. Amer. Chem. Soc., 46 (2):2066-2069.

Kramer, C. Y., 1956. "Extension of Multiple Range Tests to Group Means with Unequal Numbers of Replications," Biometrics, 12 (3): 307-310.

Leathem, J. H., and Seeley, R. D., 1947. "Plasma and Liver Protein Concentration of Rats Fed Thiouracil," Amer. Jour. Physiol., 149: 561.

_____, 1948. "Plasma and Liver Protein Concentration of Hypothyroid Rats," Endocrinology, 42:150.

Lee, N. D., and Williams, R. H., 1952. "The Role of the Pituitary-Adrenal System in Cystine -s^{35} Incorporation into Protein," Endocrinology, 51:451-456.

Ludewig, S., Minor, G. R., and Hortenstine, J. C., 1939. "Lipid Distribution in Rat Liver After Partial Hepatectomy," Proc. Soc. Exp. Biol. N. Y., 42:158-161.

Ma, T. S., and Zuazaga, G., 1942. "Micro-Kjeldahl Determination of Nitrogen An Improved Rapid Method," Ind. Eng. Chem. Anal. Ed., 14:280.

Marshak, H., and Byron, R. L., 1945. "The Use of Regenerating Liver as a Method of Assay," Proc. Soc. Exp. Biol. and Med., 59:200-202.

Mehrotra, R. M. L., and Mangalik, V. S., 1956. "An Experimental Study of the Effects of Cortisone, DOCA, and Adrenalectomy on Regeneration of Liver After Partial Hepatectomy in Albino Rats," Ind. Jour. Med. Res., 44:325-331.

Moise, T. S., and Smith, A. H., 1924. "The Effect of a High Fat Diet Upon Regeneration After Partial Hepatectomy," Jour. Exp. Med., 40:13.

Norriss, J. L., Blanchard, J., and Povolny, C., 1942. "Regeneration of Rat Liver at Different Ages," Arch. Pathol., 34:208-217.

Novikoff, A. B., and Potter, V. B., 1948. "Biochemical Studies on Regenerating Liver," Jour. Biol. Chem., 173:223-232.

Oppenheimer, M. J., and Flock, E. V., 1947. "Alkaline Phosphotase Levels in Plasma and Liver Following Partial Hepatectomy," Amer. Jour. Physiol., 149:418-421.

Ponfick, E., 1890. "Ueber Recreation der Leber," Virchows. Arch., 119:193.

Potter, V. R., and Elvehjem, C. A., 1936. "A Modified Method for the Study of Tissue Oxidations," Jour. Biol. Chem., 114:495-504.

Price, J. M., and Laird, A. K., 1950. "A Comparison of the Regenerating Liver and Induced Liver Tumors," Cancer Research, 10:650-658.

Rapport, D., Canzanelli, A., and Guild, R., 1948. "Effect of Amino Acid Deficiency on Rat Liver Before and After Partial Hepatectomy," Proc. Soc. Exp. Biol. and Med., 68:137-139.

Reid, E., 1956. "Hormonal Effects on Liver and Kidney Cytoplasm," Jour. of Endocrinology, 13:319-329.

Rerebek, J., and Rerebek, E., 1947. "The Effect of Thiouracil Upon the Cytology and Nucleic Acids of the Thyroid Gland," Acta Physiol. Scand., 14:276-288.

Robertson, W. v. B., and Kahler, H., 1942. "Riboflavin Content of Tumor Tissues," Jour. Nat. Cancer Inst., 2 (6):595-600.

Rosenthal, O., Fahl, J. C., and Vars, H. M., 1952. "Qualitative and Quantitative Changes in Alkaline Phosphatose Activity of Regenerating Rat Liver," Amer. Jour. Physiol., 171:604-611.

Schneider, W. C., 1945. "Phosphorus Compounds in Animal Tissues," Jour. of Biol. Chem., 161:293-303.

Silva, C., 1950. "Hepatic Regeneration: Action of Thiourea and Urethan," Bio. Soc. biol. Santiago Ch., 8:35-36.

Stowell, R. E., 1948. "Nucleic Acids and Cytologic Changes in Regenerating Rat Liver," Arch. Path., 46:164-178.

Strandskov, F., and Wyss, O., 1946. "The Inhibition of Bacteria by the Thiopyrimidines," Jour. Bact., 52:575-579.

Sutherland, A., 1956. "Regeneration in the Fatty Liver of the Rat After Partial Hepatectomy," Jour. of Path. and Bact., 71:403-408.

Suzuki, N., 1953. "Effect of Methylthiouracil Administration on the Nuclei Acids in the Rabbit Thyroid," Jap. Jour. Physiol., 3 (4):279-283.

Swift, H. H., 1950. "The Desoxyribosenucleic Acid Content of Animal Nucleic," Physiol. Zoo., 23:169-198.

Taleisnik, S., and Flores, N. A., 1948. "Hipertrofia Hepatica Compensadora Consecutiva a la Hepatectoma Parcial en el Sapo, Bufo Arenarum Hensel," Rev. Soc. Argentina Biol., 24:166-173.

Thomson, R. Y., Heagy, F. C., Hutchison, W. C., and Davidson, J. N., 1953. "The DNA Content of the Rat Cell Nucleus and Its Use in Expressing Results of Tissue Analysis With Particular Reference to the Composition of Liver Tissue," Biochem. Jour., 53:460.

Tipton, S. R., Major, C. W., and Smothers, J. L., 1956. "Thyroid and Adrenal Effects on Liver Protein and Enzyme Activity in Cell Fractions After Partial Hepatectomy in Rats," Proceedings XX International Physiol. Congress, p. 890.

Trotter, W. R., 1949. "Effect of Thiouracil and Uracil on the Germination of Cress Seeds," Nature, 164:63.

Welch, A. D., 1957. "The Effect of Antipyrimidines on Nucleic Acid Metabolism," Texas Reports on Biol. and Med., 15:194-198.

Wolff, R., 1948. "Sur le Mode d' Action du Thiouracile sur la Croissance de Lactobacillus casei," Compt. rend., 226:968.

APPENDIX

TABLE III-A

NUCLEI COUNTS
ANALYSIS OF VARIANCE

Source	Sum of Squares	d.f.	Mean Square	F	P
Time	11.3	3	3.77	67.73	$<.01$
Treatment	1.6	2	0.80	14.39	$<.01$
Interaction	0.2	6	0.03	b	Not sig.
Subtotal	13.1	11			
Within	2.3	39 a = 3	0.059		
Residual	2.5	45	0.050		
Total	15.4	53			

a is number of dummy replications corrected for in d.f.

b is fractional F value (not computed).

TABLE IV-A

DESOXYRIBONUCLEIC ACID PHOSPHORUS
ANALYSIS OF VARIANCE

Source	Sum of Squares	d.f.	Mean Square	F	P
Time	0.95	3	0.32	7.965	<.01
Treatment	0.04	2	0.02	b	Not sig.
Interaction	0.01	6	0.002	b	Not sig.
Subtotal	1.00	11			
Within	1.86	42 a = 1	0.044		
Residual	1.87	47	0.045		
Total	2.86	53			

a is number of dummy replications corrected for in d.f.

b is fractional F value.

TABLE V-A

RIBONUCLEIC ACID PHOSPHORUS
ANALYSIS OF VARIANCE

Source	Sum of Squares	d.f.	Mean Square	F	P
Time	26.3	3	8.77	67.98	<.01
Treatment	1.0	2	0.50	3.87	<.05
Interaction	0.3	6	0.05	b	Not sig.
Subtotal	27.6	11			
Residual	6.2	41 a = 1	0.13		
Within	5.9	47	0.14		
Total	33.5	53			

a is number of dummy replications corrected for in d.f.

b is fractional F value.

TABLE V-B

RNAP (Grams x 10^{-12}) PER NUCLEUS AT OPERATION

Experiment	Treatment		
	Propylthiouracil	Desiccated Thyroid	Control
1	3.226	2.845	2.920
2	2.780	2.983	2.718
3	2.338	2.450	1.889
4	2.726	2.810	2.461
5	2.349	2.505	2.302
6	2.932	2.737	2.613
7	3.235	2.955	2.416
8	3.317	2.412	2.406
9	3.043	2.436	2.535
Mean	2.883	2.681	2.473

ANALYSIS OF VARIANCE

Source	Sum of Squares	d.f.	Mean Square	F	P
Treatments	0.747	2	0.374	7.479	$<.01$
Experiments	1.368	8	0.171	3.424	$<.05$
Error	.799	16	0.050		
Total	2.914	26			

TABLE VI-A

PROTEIN PER CELL
ANALYSIS OF VARIANCE

Source	Sum of Squares	d.f.	Mean Square	F	P
Time	570,418	3	190,139	16.4	<.01
Treatments	257,176	2	128,588	11.1	<.01
Interaction	56,319	6	9,387	--	Not sig.
Subtotal	883,913	11			
Within	1,091,188	93 a = 3	11,733		
Residual	1,147,507	99	11,591		
Total	1,975,101	107			

a is number of dummy replications corrected for in d.f.

TABLE VI-B

PROTEIN (Grams x 10^{-12}) PER NUCLEUS AT OPERATION

Experiment	Treatment		
	Propylthiouracil	Desiccated Thyroid	Control
1	825.8	540.9	590.4
	735.5	542.4	609.6
2	708.1	625.2	724.1
	730.4	641.9	716.5
3	711.3	587.7	621.3
	761.4	565.8	602.0
4	648.3	504.3	605.8
	595.9	550.7	574.6
5	598.1	584.1	607.5
	605.7	601.9	615.4
6	775.4	666.5	709.3
	777.4	654.3	--
7	808.7	653.5	658.8
	815.7	627.6	688.9
8	842.7	556.5	639.3
	921.8	553.6	619.5
9	686.4	548.0	671.8
	716.0	539.0	--
Mean	736.9 **	585.8 *	646.4

*Indicates $P < .05$ for means between which it is located.

**Indicates $P < .01$ for means between which it is located.

TABLE VI-C

PROTEIN PER CELL AT OPERATION
ANALYSIS OF VARIANCE

Source	Sum of Squares	d.f.	Mean Square	F	P
Treatments	208,000	2	104,000	17.70	$<.01$
Experiments	109,000	8	13,625	2.32	Not sig.
Interaction	94,000	16	5,875	7.34	$<.01$
Subtotal	411,000	26			
Within	20,000	25 a = 2			
Total	431,000	53			

a is number of dummy replications corrected for in d.f.

TABLE VII-A

PERCENTAGE DRY WEIGHT
ANALYSIS OF VARIANCE

Source	Sum of Squares	d.f.	Mean Square	F	P
Time	251	3	83.66	24.8	<.01
Treatment	13	2	6.50	1.92	Not sig.
Interaction	28	6	4.67	1.47	Not sig.
Subtotal	292	11			
Within	124	39 a = 3	3.18		
Residual	152	45	3.38		
Total	416	53			

a is number of dummy replications corrected for in d.f.

TABLE VIII-A

ACID SOLUBLE PHOSPHORUS
ANALYSIS OF VARIANCE

Source	Sum of Squares	d.f.	Mean Square	F	P
Time	105,000	3	35,000	3.91	<.05
Treatment	54,000	2	27,000	3.01	Not sig.
Interaction	29,000	6	4,833	b	Not sig.
Subtotal	188,000	11			
Within	401,000	42 a = 1	9,548		
Residual	430,000	48	8,958		
Total	589,000	54			

a is number of dummy replications corrected for in d.f.

b is fractional F value (not computed).

TABLE IX-A

PHOSPHOLIPID PHOSPHORUS
ANALYSIS OF VARIANCE

Source	Sum of Squares	d.f.	Mean Square	F	P
Time	1,554,000	3	518,000	13.28	$<.01$
Treatment	94,000	2	47,000	1.21	Not sig.
Interaction	101,000	6	16,833		Not sig.
Subtotal	1,749,000	11			
Within	1,537,000	36 a = 4	42,694		
Residual	1,638,000	42	39,000		
Total	3,286,000	51			

a is number of dummy replications corrected for in d.f.

TABLE X-A

PER CENT RESTORATION OF COMPONENTS BETWEEN TWENTY-FOUR AND FORTY-EIGHT HOURS ANALYSIS OF VARIANCE

Source	Sum of Squares	d.f.	Mean Square	F	P
Compounds	5,082	3	1,694	21.2	<.01
Treatments	447	2	224	2.8	Not sig.[a]
Time	7,085	1	7,085	88.8	<.01
Treatments x compounds	294	6	49	0.6	Not sig.
Treatments x time	498	2	249	3.1	Not sig.[a]
Time x compounds	504	3	168	2.1	Not sig.
Triple interaction	176	6	29		
Replications	1,476	2			
Error	3,670	46	79.78		
Total	19,232	71			

[a]F value for significance at 5 per cent level is 3.2.