tinal mucosa would not revert to normal under the incubation procedures used here.

In Exp. C.4 with 50 ml of plasma the ATP level was preserved at a slightly higher level than in Exp. C.3 in which only 10 ml of plasma were used. This suggests that although these incubation conditions were far from ideal, the extra plasma did have some beneficial effect, perhaps by supplying a nutrient or neutralizing some toxic substance.

Discussion. Our earlier studies showed that whole blood could be incubated for several hours with no loss of ATP. Extension of these studies to other tissues was uniformly unsuccessful. In dog muscle, mincing in incubation medium caused nucleotide breakdown. In dog or rabbit intestinal mucosa, the cells could not even be scraped off into any kind of medium without deterioration of the nucleotide pattern. Injecting trichloroacetic acid into the living tissue is similar to the study of Dietrich and Friedland(3) which reported *in vivo* injection of perchloric acid into rabbit intestine. Their AMP:ADP:ATP ratios of 0.10:0.31:1.28 were not greatly different from ours, but perhaps both results are only approximations to the true *in vivo* distribution.

Summary. Our experiments indicate that the nucleotides of different tissues vary greatly in lability. When dog muscle was removed rapidly and minced in ice cold trichloroacetic acid, its nucleotide pattern resembled that seen in fresh blood, *i.e.*, ATP greatly exceeded ADP or AMP. Delay in mincing the tissue caused some decrease in ATP with increases in ADP and AMP. On the other hand, dog or rabbit intestinal mucosa was so labile that usual procedure of removing tissue and transferring it immediately to cold trichloroacetic acid was much too slow. Injecting cold trichloroacetic acid into the live tissue was necessary to give a nucleotide pattern which resembled patterns seen in other tissues. Incubation under circumstances tried, were unsuccessful in restoring tissue nucleotide patterns. Extrapolation of foregoing results suggests that studies involving either distribution of nucleotides in tissues or determination of tissue nucleotides after *in vitro* incubation must indicate degree of lability of these tissue nucleotides.

1. Bishop, C., Rankine, D. M., Talbott, J. H., *J. Biol. Chem.*, 1959, v234, 1233.

2. Umbreit, N. W., Burris, R. H., Stauffer, J. F., (eds.), *Manometric Techniques*, 3rd Edit., Minneapolis, Burgess, 1957, p149.

3. Dietrich, L. S., Friedland, I. M., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 470.

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Effect of Thyroidectomy on Development of Polyploid Nuclei in Rat Liver.*† (25977)

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The role of growth hormone in the appearance and maintenance of polyploid cells in normal liver was originally described by Helweg-Larsen(1) and Leuchtenberger *et al.*(2) and confirmed and extended by DiStefano and Diermeier(3) and Geschwind *et al.*(4). Recently, involvement of 2 other hormonal systems has become apparent. The gonadal hormones have been implicated as a result of a study of the effect of castration on polyploidization of rat liver(5) and, as indicated by Carriere in preliminary reports(6,7), thyroid hormone is also involved. The role of the latter has been further elaborated upon in recent experiments(8) on effects of thyroxin

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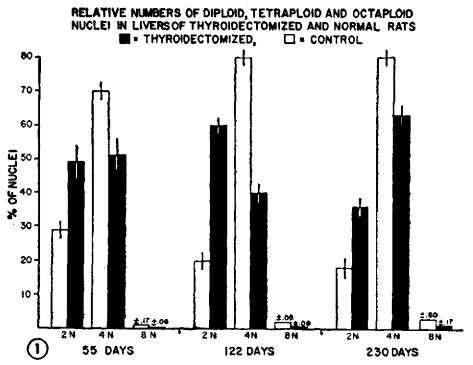
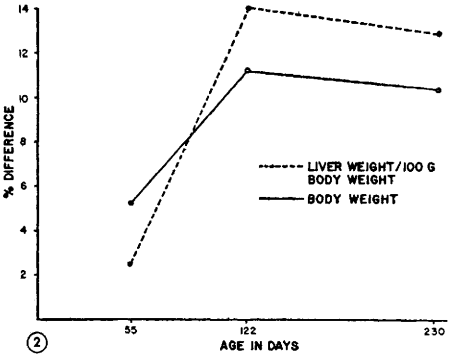
and growth hormone on ploidy development in livers of dwarf mice and hypophysectomized rats. The present study confirms the effect of thyroidectomy on polyploidization of the rat liver and, since it reveals a unique effect of partial thyroid deprivation or its equivalent, extends the observations already made. It also indicates the necessity of extended-time studies when dealing with problems of developmental ploidy in rat liver, and attempts to elaborate upon the mechanics of ploidy as a growth phenomenon. It will also be demonstrated that polyploid composition of the liver appears to be a more sensitive indicator of thyroid deficiency than growth of the animal as proposed by Evans *et al.*(9).

Materials and methods. Sixteen females from 9 litters of Sprague-Dawley rats were thyroidectomized at 21 days of age, 13 unoperated littermates serving as controls. The animals were fed *ad libitum* on a standard Purina diet and 1% calcium gluconate. Weekly body weight determinations were made and tissues were taken at 55, 122, and 230 days of age. At these times total liver weights were obtained for all animals, and tail, body, and humerus lengths for the 122-day group. Blocks of liver were removed from the center of the left lateral lobe, fixed in Lavdowsky's fluid and sectioned at 10 μ. As control on completeness of thyroidectomy, tracheas and adjacent muscle masses were sectioned, and every tenth section examined. A few residual follicles were found in 3 of the animals but they were considered to be completely thyroidectomized since their liver cytology was consistent with that of the other operated animals. Since pituitary acidophil degranulation follows thyroidectomy(10,11), the hypophyses of the 122-day operated and control animals were preserved and stained with aldehyde fuchsin to determine effectiveness of the operation. For the purpose of this paper, binuclearity was ignored, and polyploidy considered only from the standpoint of DNA content per nucleus. Establishment of DNA classes was based on 30 cytophotometric determinations per liver as previously described(5). This information was used as the basis for visual classification of 2000 nuclei per liver, 1000 by each of 2 trained observers working independently on coded slides (5).

Results. Because the animals were maintained on a normal diet we believe that our data represent the result of initial extreme thyroid deficiency followed by either thyroxin replacement from hypertrophying accessory tissue or gradual supplementation by dietary iodide and/or thyroxin. Thus, the recent work of Evans *et al.*(9), indicating that levels of iodide sufficient to keep pituitary acidophils active might conceivably be found in a normal diet, can explain, in part, the results obtained below. While it has been observed that the experimentals, at all age levels, have obviously fewer polyploid nuclei than littermate controls, for 6 of the animals a small percentage of octaploid nuclei were counted by the visual method, while none with the octaploid amount of DNA could be found by cytophotometry. This indicates a certain number of nuclei have increased in volume without DNA increase, and suggests interference with DNA synthesis in the thyroidectomized rats. It also warns against the assumption that nuclear volume is consistently related to degree of ploidy.

Fig. 1 summarizes, by age group, the results of visual classification of polyploid nuclei. These nuclei, absent at birth, increase in number with age so that at time of weaning (day of operation in these experiments) the livers contain 90% diploid (2N), 10% tetraploid (4N), and virtually no octaploid (8N) nuclei. Stability for normal animals is reached by 122 days of age, at which time the ploid composition is approximately 18-20% 2N, 80% 4N, and 1-2% 8N. At 55 days of age formation of ploid nuclei in experimental livers has progressed beyond the level at time of operation, but lags significantly behind the control group. Nevertheless, livers of the thyroidectomized animals are predominantly 4N. At 122 days of age the ploid population has "regressed" so that livers of the experimental group contain only 40% 4N nuclei with a correspondingly smaller percentage of 8N nuclei. Since the livers were growing between 55 and 122 days this "regression" might also be interpreted as a retarded formation of polyploid nuclei during continued production of diploid nuclei. In either event, the liver





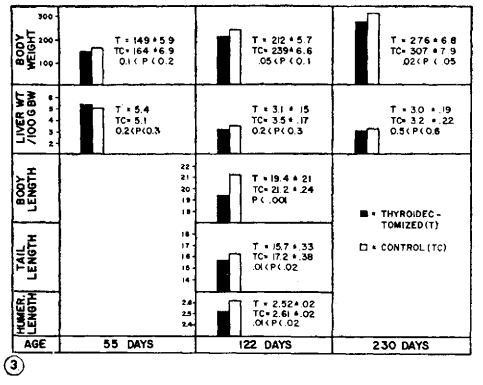


FIG. 1. % composition of polyploid nuclei, by age group, in livers of normal and thyroidectomized rats (operated on at 21 days). Stand. errors indicated by vertical lines.

FIG. 2. % differences between relative liver weights of normal and thyroidectomized rats, and between body weights of the same animals.

FIG. 3. Mean body and relative liver weights, and tail, body and humerus lengths for normal and thyroidectomized rats, arranged by age group. P values determined from t test for small samples.

cell appears to be more sensitive to thyroid deficiency at 122 days than at 55 days, since there is no reason to believe that thyroidectomized animals are any more thyroid-deficient at 122 days than at 55. In fact, as shown below, there is good evidence that they are less thyroid deficient at 122 days than at 55. Alternatively, the polyploid status at 122 days may only reflect a delayed response to a much earlier stimulus, although most of the data gathered thus far would lead one to expect a faster response to hormonal imbalance. At 230 days the situation has again reversed so that the operated animals appear to be approaching the controls cytologically, although there is still a significant difference at all ploid levels.

The polyploid regression appears similar to that following hypophysectomy and, because pituitary acidophil degranulation follows thyroidectomy, can be most satisfactorily explained by diminished growth hormone output. Furthermore, the sequence of events as described above is quite consistent with observed growth patterns and pituitary cytology. Although no counts were made in pituitaries of the 122 day group, we have observed large numbers of granulated acidophils in all specimens. If the assumption can be made that complete acidophil degranulation followed thyroidectomy(10,11) it is evident that extensive acidophil regranulation has occurred in all of the experimental pituitaries. Considering the evidence cited by Geschwind *et al.* (8), reactivation of acidophils in presence of dietary iodide or thyroxin is an acceptable possibility. Since the liver appears to exhibit marked sensitivity to lack of thyroxin at the same age at which regranulated acidophils are observed, appearance of the latter must, for the present, be considered as representing the beginning of acidophil reactivation.

Growth differences for the 3 age groups support the concept of replacement of iodide and/or thyroxin from either dietary or endogenous sources, which is strikingly reflected after 122 days (Fig. 2). For the 2 parameters recorded the differences are least at 55 days, maximum at 122 days, and slightly less at 230 days, indicating a tendency for operated animals to approach the growth of normals at 230 days. While the decrease between 122 and 230 days is slight it is significant that, for each of the litters studied, the change is consistently in the same direction.

Since polyploidization is a liver growth process characteristic of postnatal growth of the organ, we have attempted to analyze upon what other growth factors, if any, the process is dependent. Absolute and relative liver weights and liver growth rate have been examined as possible determinants. At 122 days, the age of maximum liver effect, mean liver weight for operated animals is 6 g, equivalent to mean liver weight of 55 day normal rats. Comparison of ploidy status in the 6 g liver of the 122 day thyroidectomized rat and the 6 g liver of the 55 day normal rat (Fig. 1) reveals that the 2 are dissimilar, and that polyploid percentages in the operated animals are characteristic of normal animals younger than 55 days. Fig. 3 is a summary of mean values of several growth parameters recorded for the 3 age groups. Examination of relative liver weights reveals a decrease with age in normal animals, declining from approximately 13 at birth to about 3.5 from 122 days. Since polyploidization of normal liver occurs coincidentally with decrease in relative weight, it might be assumed that an inverse ratio exists between it and polyploid status. However, examination of P values reveals no significant difference between operated and control relative liver weights at any age level, although polyploid composition shifts significantly (Fig. 1). Furthermore, significant polyploid shifts can still be observed in operated rats once relative liver weights have reached stability. With respect to liver growth rate we have previously shown, in the castrate female, that growth rate of both body and liver is accelerated, while formation of 8N cells is retarded(5). Therefore, it appears that absolute and relative liver weight, and liver growth rate, while apparently coordinated with polyploid development in the normal rat, are not positively related and cannot be considered as determinants for polyploid composition of the liver.

Fig. 3 further reveals that body weight difference between thyroidectomized and control animals becomes increasingly significant with age, and differences in body, tail and humerus lengths are quite significant at 122 days, but not as great as one would expect from animals thyroidectomized and injected with I^{131} or maintained on iodine-free diets. However, since the polyploid shifts approach the expected maximum, the experimental conditions have served to demonstrate that polyploid composition of liver is a more sensitive indicator of thyroid deficiency than the usually recognized growth parameters.

Summary. Thyroidectomy of weanling rats kept under normal dietary conditions results in initial prolonged retardation in production of polyploid nuclei in the liver, followed by gradual resumption of polyploid cell formation over long periods. The effect appears to be mediated, at least in part, through the pituitary, and polyploid shifts are greater than can be explained on the basis of absolute or relative liver weight, or liver growth rate. The liver cytology appears to be more sensitive to thyroid deficiency than the general growth of the animal, the time of greatest sensitivity for this study having been observed at 122 days of age.

1. Helweg-Larsen, H. F., *Acta Path. Scand.,* 1952, Suppl. 92.

2. Leuchtenberger, C. L., Helweg-Larsen, H. F., Murmanis, L., *Lab. Invest.,* 1954, v3, 245.

3. DiStefano, H. S., Diermeier, H. F., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 590.

4. Geschwind, I. I., Alfert, M., Schooley, C., *Exp. Cell Res.,* 1958, v15, 232.

5. Swartz, F., Sams, B. F., Barton, A. G., *ibid.,* in press.

6. Carriere, R., *Anat. Rec.,* 1955, v121, 273.

7. ———, *Rev. Can. de Biol.,* 1957, v16, 529.

8. Geschwind, I. I., Alfert, M., Schooley, C., *Biol. Bull.,* 1960, v118, 66.

9. Evans, E. S., Rosenberg, L. L., Simpson, M. E., *Endocrinology,* 1960, v66, 433.

10. Purves, H. D., Griesbach, W. E., *Brit. J. Exp. Path.,* 1946, v27, 170.

11. Knigge, K. M., *Anat. Rec.,* 1958, v130, 543.

Received May 19, 1960. P.S.E.B.M., 1960, v104