The test conditions were described previously[4]. 5-HT was used as creatinine sulfate (generously supplied by Abbott Laboratories); the doses refer to the base.

5-Hydroxytryptamine in Mammalian Thyroid Glands

									Mean µg/g*
Rat	2·5	2·6	2·4	3·3	5·0	5·0	3·9	4·6	3·7
Sheep	3·5	5·7	7·5	3·8	2·4				4·6
Dog	0·025	0·018	0·038	0·02					0·025
Rabbit	0·29	0·09							0·19

* Wet weight.

The 5-HT equivalents are presented in the Table. The concentrations found in the rat and sheep thyroids were more than hundred times those found in the dog thyroids. Rabbit thyroids contained more than those of dogs. In order to ascertain the specificity of these findings, the following studies were carried out.

(1) Ascending paper chromatograms were run using the rat and sheep thyroid extracts in the upper organic phase of *n*-butanol/glacial acetic acid/water (4:1:5). When pieces of the paper strips were eluated and tested on *Venus* heart, the active material was found to travel like 5-HT. When paper chromatograms of sheep thyroid extract were treated according to JEPSON and STEVENS[5], a fluorescing spot was found at the same site as the spot of the reference 5-HT.

(2) Parallel assays were done with rat and sheep thyroid extracts on the atropinised estrus rat uterus[3] and *Venus* heart. Sheep extracts gave about one third lower results on the uterus. The reason for this is supposed to be the presence of catechol amines, which relax the uterus. In one sheep thyroid gland 0·2 µg/g of norepinephrine and 0·03 µg/g of epinephrine were found when analysed according to v. EULER[6]. The rat extracts, on the other hand, were about 30% more active on the uterus than on the heart. LSD treatment did not completely abolish the response of the uterus to rat thyroid extracts, although it blocked the effect of 5-HT. There was little, if any, sign of this LSD-resistant activity in sheep and dog thyroid extracts. The low amount of 5-HT in the dog thyroid gland was also confirmed on the uterus. The amounts of norepinephrine and epinephrine in one dog thyroid were 0·3 and 0·04 µg/g respectively.

(3) That the activity measured on *Venus* heart was due to 5-HT is strengthened by the similar time course of the response. After washing, the effect of bufotenine, and, to a much lesser degree, the effect of N-methyl-5-HT, lasts longer than the effect of 5-HT. The molluscan heart is resistant to most substances likely to have an effect on smooth muscle[7].

(4) The rat thyroid extract did not lose its activity on the *Venus* heart when boiled for 10 min in 1 *N* HCl, while the same treatment in 1 *N* NaOH destroyed about 80%.

(5) Reserpine (5 mg/kg intraperitoneally) was not able, within 4 h, to decrease significantly the *Venus* heart activity of rat thyroid glands; however, neither was their a clear-cut 5-HT depletion in the duodenum of these rats.

For comparison some salivary glands were extracted and their activity tested on the *Venus* heart. In the rat rather high values were found (in µg/g, parotid: 0·68, 0·44; submaxillary: 0·19, 0·31; sublingual: 0·61, 1·0; extraorbital lacrimal: 0·34, 0·26). In the dog, the values were again lower (parotid: 0·03, 0·005; submaxillary; 0·02, 0·004); and in the rabbit, they were between those of the rat and the dog (parotid: 0·16; submaxillary: 0·13).

Conclusions: High 5-HT values were found in rat and sheep thyroid glands and very low values in dog thyroids. In the rat thyroid, there appears to be an unidentified uterus-stimulating substance which is soluble in 90 % acetone. On rat uterus, it is atropine-resistant. It is not histamine, because the tissues used for analysis are resistant to this amine. GARVEN[8] tested two acetone extracts of rabbit thyroid on the rat uterus and found one of them to produce an LSD-resistant response. The smooth muscle-stimulating substance of KOEPF and MEZEN[9], which is present in rat salivary glands, also was found to contract the rat uterus. It is not known where 5-HT is localized in the mammalian thyroid gland. The mast cells are a possible site, since they are known to contain this amine[10]; certain cells of the enterochromaffin type are another possible location. It is improbable that 5-HT has anything to do with the specific thyroid function.

It is a pleasure to thank Professor O. KRAYER for making my visit to his department possible and for his interest in this work. Helpful advise by Professor V. ERSPAMER is gratefully acknowledged.

M. K. PAASONEN*

Department of Pharmacology, Harvard Medical School, Boston, Mass., September 13, 1957.

Zusammenfassung

Schilddrüsen von Ratten und Schafen enthalten relativ grosse Mengen von 5-Hydroxytryptamin. In der Thyreoidea von Hunden wird dieser Stoff nur in einer hundertmal kleineren Konzentration vorgefunden. Weiter wird gezeigt, dass in der Rattenthyreoidea eine nicht identifizierte, acetonlösliche Substanz vorhanden ist, welche Uteruskontraktionen auszulösen vermag.

[4] M. K. PAASONEN and M. VOGT, J. Physiol. *131*, 617 (1956).
[5] J. B. JEPSON and B. J. STEVENS, Nature *172*, 772 (1953).
[6] U. S. v. EULER, Arch. int. Pharmacodyn. *77*, 477 (1948).
[7] B. M. TWAROG and I. H. PAGE, Amer. J. Physiol. *175*, 57 (1953). J. H. GADDUM and M. K. PAASONEN, Brit. J. Pharmacol. *10*, 474 (1955).
[8] J. GARVEN, Brit. J. Pharmacol. *11*, 66 (1956).
[9] G. F. KOEPF and J. F. MEZEN, J. Pharmacol. *60*, 407 (1937).
[10] E. P. BENDITT, R. L. WONG, M. ARASE, and E. ROEPER, Proc. Soc. exp. Biol., N. Y. *90*, 303 (1955).
* Present address: Department of Pharmacology, University of Helsinki (Finland).

Fat Metabolism in Experimental Obesities VIII. Blood Total Lipids and Ketones in Four Kinds of Obese Mice*

To our knowledge, no systematic study has been conducted of blood lipids and ketone levels in various

* Supported in part by Grants-in-Aid from the National Institute of Arthritis and Metabolism (Grant No. A–49) National Institutes of Health, Public Health Service, Bethesda, Md., Nutrition Foundation Inc., New York; Albert and Mary Lasker Foundation, New York; and the Fund for Research and Teaching, Dept. of Nutrition.

Table I

Blood Glucose of Non-Obese Mice and of Mice with Four Different Obesities (mg%)

	Hereditary Obese-Hyperglycemic and Controls		Goldthioglucose Obese and Controls		Yellow Obese and Controls		Adrenotropic Tumor Obese and Controls	
	Obese	Non-Obese	Obese	Non-Obese	Obese	Non-Obese	Obese	Non-Obese
Fed – No treatment . .	286 ± 93	127 ± 10	157 ± 31	114 ± 21	151 ± 32	130 ± 12·1	108 ± 10	128 ± 3
After 18 h fast	128 ± 36	62 ± 11	97 ± 13	87 ± 18	78 ± 29	85 ± 24	115 ± 13	77 ± 8
After 0·5 units Insulin	215 ± 90	35 ± 14	49 ± 12	52 ± 9				
After 2 × 2 mg growth hormone	382 ± 60	125 ± 18	167 ± 42	134 ± 17				

experimental obesities. Previous studies published in this series (e.g.[1]) and elsewhere (reviewed in [2]) have emphasized the differences between 'regulatory' obesities, characterized by central lesions in the mechanism regulating food intake, and 'metabolic' obesities, characterized by primary disturbances in fat metabolism. For instance, in the first type (exemplified by surgically-induced and by goldthioglucose-induced hypothalamic obesity) increased lipogenesis takes place only in the measure in which an increase in food intake is allowed. In the second type (exemplified by the obese hyperglycemic syndrome and by the obesity due to ACTH-secreting tumors), lipogenesis is increased over the normal even if the obese animals are paired-fed with their non-obese controls or if both groups are fasted[3]. In turn, different metabolic obesities will differ in the nature of the primary metabolic abnormality. For instance, obese-hyperglycemic mice have very different endocrine, enzymatic, metabolic and behavioral characteristics from ACTH-obese mice[2]. It appeared of interest to see whether the blood lipid and ketone picture would show a similar diversity.

Materials and Methods.—The mice used belong to four kinds of obesity available at this time for this species in our laboratory[4]. Mice with the recessive hereditary obese-hyperglycemic syndrome[2], ranged in age from 3 to 7 months, weighed from 40 to 90 g for the obese, 19 to 33 g for their non-obese littermates. No differentiation is made in the tables of results on the basis of sex for these and other types of mice as no difference was detected between males and females as regards total lipids or ketone bodies. Goldthioglucose hypothalamic obese swiss mice[5] had been successfully injected with 1 mg/g of goldthioglucose 3 to 5 months prior to the experiment. Controls were swiss mice unsuccessfully injected or left uninjected, no difference being detected between the two groups. Obese and non-obese mice in the goldthioglucose group were 7 to 9 months of age; the obese animals weighed 50 to 70 g, non obese 30 to 40 g. Adrenotropic-tumor bearing mice[6], were six months old and had received the tumor transplant two months previously. The 'obese' animals weighed on the average 30 g, the non obese 27 g (in this type, there can be considerable increase in proportion of bodyweight represented by fat without any marked increase in weight[7], in other words 'obesity' is present even in the absence of 'overweight'). ACTH animals, being highly susceptible to infections, were given terramycin in their drinking water (concentration 2 g per 1000 cm³). Mice with the dominant heterozygous yellow obesity[8] were 4–6 months of age and weighed 40 to 60 g; their non-obese littermates weighed 24 to 30 g. All animals were housed in pairs (one obese, one non-obese) in small cages for at least two weeks preceding the period when determinations were made and throughout that period they received food and water *ad libitum* except for 'fasting' experiments when the animals were deprived of food 18 h prior to the determination. In addition, in the obese hyperglycemic mice, as detailed studies had previously been conducted in lipogenesis following the administration of various hormones to these animals[9], the effects of growth hormone and of insulin on total lipids and ketone levels were also determined. The insulin was given intraperitoneally, a dose of 0·5 units being given in 0·25 cm³ aqueous solution, the samples being taken 30 min after the administration of the hormone. Growth hormone was also given intraperitoneally, each dose of 2 mg being dissolved in 0·25 cm³ of saline at a pH of 8·5. Each mouse received two doses in two consecutive days, the samples being drawn 15 h after the second injection. Each animal acted as its own control, with no less than 10 days elapsing between experiments. Blood samples were drawn from the tail vein. Glucose levels were determined by the SOMOGYI-NELSON micromethod[10]. The blood lipid levels were determined by the turbidimetric method of DE LA HUERGA, YESNECK, and PAPPER[11]. Ketone bodies were determined as acetone and acetoacetic equivalent by the colorimetric method of GREENBERG and LESTER[12].

Results and Discussion.—Table I gives blood glucose levels of the animals under study and confirms previous findings on blood glucose of these various types of obese

[1] M. W. BATES, C. ZOMZELY, and J. MAYER, Amer. J. Physiol. *181*, 187 (1955). – J. MAYER, N. C. HAGMAN, N. B. MARSHALL, and A. J. STOOPS, Amer. J. Physiol. *181*, 501 (1955).

[2] J. MAYER, Nutr. Abstr. Rev. Part I and Part II *25*, 597 (1955); *25*, 871 (1955).

[3] J. MAYER and C. Y. ZIGHERA, Exper. *11*, 358 (1955).

[4] We are endebted to Schering Corporation for a gift of goldthioglucose.

[5] N. B. MARSHALL, R. J. BARRNETT, and J. MAYER, Proc. Soc. exp. Biol. Med. *90*, 240 (1955).

[6] Obtained through the kindness of Dr. J. FURTH, Children's Cancer Foundation, Boston. – J. MAYER, C. ZOMZELY, and J. FURTH, Science *123*, 184 (1956). – K. H. SHULL, J. ASHMORE, and J. MAYER, Arch. Biochem. Biophys. *62*, 210(1956). – C. ZOMZELY and J. MAYER, Amer. J. Physiol. *187*, 365 (1956).

[7] J. MAYER, C. ZOMZELY, and J. FURTH, Science *123*, 184 (1956).

[8] Bred from animals kindly given by Dr. PAUL FENTON, Brown University, Providence, Rhode Island. – J. MAYER, Physiol. Rev. *33*, 472 (1953). – K. J. CARPENTER and J. MAYER, Amer. J. Physiol. (in press).

[9] D. J. SILIDES and J. MAYER, Exper. *12*, 66 (1956).

[10] M. SOMOGYI, J. biol. Chem. *160*, 61 (1945).

[11] J. DE LA HUERGA, C. YESENICK, and H. PAPPER, Amer. J. clin. Pathol. *23*, 1163 (1953).

[12] L. A. GREENBERG and D. LESTER, J. biol. Chem. *154*, 177 (1944).

Table II

Blood Total Lipids, Acetone and Aceto Acetic Acid Equivalent of Non-Obese Mice and of Mice with Four Different Obesities

Type of Animal and Treatment	Total Lipids		Acetone		Aceto Acetic Acid	
	Obese	Non-Obese	Obese	Non-Obese	Obese	Non-Obese
Obese Hyperglycemic Fed — No Treatment	955 ± 239 (12)	632 ± 148 (12)	3·17 ± 1·49 (15)	2·03 ± 0·79 (15)	3·48 ± 1·64 (15)	3·25 ± 0·88 (15)
Obese Hyperglycemic Fasted 18 h	1255 ± 364 (10)	1105 ± 259 (8)	2·30 ± 1·37 (15)	3·59 ± 0·40 (13)	2·53 ± 1·03 (13)	3·75 ± 0·47 (13)
Obese Hyperglycemic ½ I. U. Insulin per Mouse	1221 ± 114 (10)	1246 ± 236 (8)	2·57 ± 1·38 (10)	2·99 ± 2·11 (9)	2·82 ± 1·50 (10)	3·29 ± 2·29 (9)
Obese Hyperglycemic 2 mg Growth Hormone per Mouse for 2 Days	1598 ± 199 (9)	1631 ± 82 (6)	3·28 ± 1·99 (11)	2·85 ± 1·59 (10)	3·60 ± 2·31 (11)	3·13 ± 1·74 (10)
G. T. G. Fed	1214 ± 55 (6)	872 ± 71 (6)	1·70 ± 0·38 (16)	1·50 ± 0·25 (13)	1·87 ± 0·31 (16)	1·64 ± 0·28 (13)
G. T. G. Fasted 18 h	1756 ± 32 (6)	1313 ± 47 (6)	1·94 ± 0·61 (15)	2·18 ± 1·01 (10)	2·13 ± 0·67 (15)	2·32 ± 1·10 (10)
Yellow Fed	1517 ± 76 (8)	1174 ± 57 (6)	3·68 ± 1·54 (16)	1·39 ± 0·39 (11)	4·05 ± 1·68 (16)	1·53 ± 0·44 (11)
Yellow Fasted 18 h	1763 ± 92 (8)	1597 ± 64 (6)	2·04 ± 0·66 (11)	1·97 ± 1·15 (10)	2·28 ± 0·79 (11)	2·14 ± 1·16 (10)
A. T. T. Fed	1806 ± 50 (5)	1397 ± 134 (5)	1·24 ± 0·13 (6)	1·49 ± 0·40 (4)	1·36 ± 0·18 (6)	1·64 ± 0·53 (4)

animals (reviewed in [2]). The mice with the hereditary obese hyperglycemic syndrome show a very much elevated blood glucose—in this series over twice the level of the non-obese. This high level is relatively little affected by insulin, further elevated by growth hormone. By contrast, the blood glucose level of goldthioglucose obese mice, like that of normal mice, is almost unaffected by growth hormone under the conditions of the experiment, and decreased to such levels by insulin that convulsions appear in some of the animals. The non-fasted glucose level of yellow mice is elevated in some of the animals. Unlike that of normal mice the blood glucose level of mice bearing adrenotropic tumors is relatively unaffected by fasting.

Table II gives the blood lipids of the various groups of animals. It is of interest that the four strains of non-obese mice have markedly different levels of blood total lipids. On the other hand, all these animals react similarly to fasting: as could be predicted, fasting elevates lipid levels; in conformity with classical results, insulin and growth hormone cause an increased lipemia in the thin (normal) littermates of the hereditarily obese-hyperglycemic mice. In each of the four types of mice studied, blood total lipid levels are higher in the obese than in the non-obese mice. In the three types of obese mice in which the effect of fasting was determined, it led to a significant increase in total blood lipids. In the obese hyperglycemic mice, the various treatments tend to make levels similar to these of the control animals.

Of particular interest are the results concerning blood acetone and aceto-acetic acid levels. In the non-obese animals, in accordance with classic results, fasting causes a highly significant increase in ketone levels. The goldthioglucose obese mice (exemplifying 'regulatory obesity') behave as do normal animals and exhibit increased ketone levels in fasting. By contrast, in the hereditarily obese-hyperglycemic mice (exemplifying 'metabolic obesity') fasting decreases ketone levels. It thus appears that reaction of ketone levels to fasting may provide an additional method of distinguishing between 'regulatory' and 'metabolic' obesities. Yellow obese animals follow a pattern similar to that exhibited by the obese hyperglycemic mice, blood ketone levels being cut in half by fasting as compared to the expected rise in the non-obese littermates.

In connections with the last finding it may be relevant to recall that while the blood cholesterol level of goldthioglucose obese mice is normal[2], that of mice with the obese-hyperglycemic syndrome[13], of mice made obese by ACTH secreting tumors[14] and of yellow obese mice[15] is elevated by amounts varying from 30% in the case of the yellow mice to 100% in the case of the obese hyperglycemic mice and the ACTH mice. The results of this study again point to the fact that, while there are certain non-specific results of hyperphagia and obesity (e.g. elevated serum lipids) there are also profound differences between regulatory obesity, with its near normal metabolic pattern, and metabolic obesities with their galaxies of abnormalities primary to the various syndromes (e.g. blood glucose picture, enzymatic activities, hormonal patterns, cholesterol metabolism and, in this study, the effect of fasting on blood ketones).

J. MAYER and D. J. SILIDES

Harvard University, School of Public Health, Boston (Massachusetts), October 15, 1957.

Résumé

Chez la Souris, l'obésité «de régulation» (ici: l'obésité hypothalamique due à l'aurothioglucose) comme les obésités «de métabolisme» (exemples: syndrome récessif d'obésité-hyperglycémie, syndrome dominant d'obésité «jaune», obésité due aux tumeurs secrétant l'ACTH) sont accompagnées d'hyperlipémie, accentuée par le jeûne.

[13] J. MAYER and A. K. JONES, Amer. J. Physiol. *175*, 339 (1953).
[14] C. ZOMZELY and J. MAYER, Amer. J. Physiol. *187*, 365 (1956).
[15] M. SOMOGYI, J. biol. Chem. *160*, 61 (1945).

Par contre si, comme les souris normales, les souris obèses hypothalamiques réagissent au jeûne par une augmentation de la cétonémie, les souris héréditairement obèses réagissent au jeûne par une diminution de la cétonémie, une réaction qui peut s'ajouter à celles qui différencient les obésités «de métabolisme» des obésités «de régulation».

Nuclear Chimaeras in the Newt

Direct tests as to whether the cell nuclei of various tissues differ in their developmental capacities have been made recently by BRIGGS and KING. These authors developed a technique for the transplantation into frog oocytes of somatic nuclei from embryos of various stages. The recipient oocytes are first enucleated and artificially activated, and the degree of development they reach after nuclear transfer shows the capacity of implanted nuclei to carry the oocyte cytoplasm towards normal differentiation. The above authors have reviewed their investigations recently[1]; whilst blastula nuclei, including nuclei from the dorsal lip of the blastopore, are capable of bringing about the normal development of enucleated oocytes, endoderm nuclei from late gastrula show definite signs of differentiation in that a high percentage of them are incapable of effecting development beyond the gastrula stage.

It has been found difficult to carry out parallel investigations with the oocytes of newts, as these cannot become artificially activated and are normally polyspermic. Attempts made so far[2] involved substantial modifications of the BRIGGS and KING procedure, and the results have not been entirely satisfactory.

It might be of interest to record here a further experiment carried out on *Triturus alpestris* material.

A group of 2 to 4 somatic nuclei were transplanted into a normally fertilized and non-enucleated egg. As a result, this egg carried its normal zygotic nucleus as well as the donor nuclei. It was hoped that the hosts would develop to a more or less advanced stage, at which it would be possible to observe whether the donor nuclei are able to participate in tissues derived from all three cell layers or only in tissues derived from the cell layer of their origin.

To make identification of implanted nuclei possible, haploid embryos were used as donors. These were produced by Dr. G. G. SELMAN of this Institute, who treated *in vitro* newly collected sperm with ultraviolet light of 2100–3200 Å for 1·5 to 3 min. The sperm was then smeared onto oocytes collected from the oviduct. The donors were checked for haploidy by chromosome counts on squashes from a piece of tissue.

The host eggs were left to develop after the nuclear transfer. Sixteen were fixed at the early neural plate stage, sectioned and stained with Feulgen for chromosome counts. The remaining failed to develop normally beyond the early neural plate stage, became abnormal and finally cytolysed.

Examination of the 16 specimens fixed showed that no haploid nuclei could be seen in 5; but several haploid (donor) as well as diploid (host) nuclei were found in each of the remaining 11. Of these 11, 7 had received haploid ectodermal nuclei from the neural plate itself of donor embryos whilst 4 had received nuclei from the chorda mesoderm of the same donors.

It is interesting that, in almost all of the eleven cases, nuclei of donor origin were found in all three cell layers. Thus, not only the embryos as a whole but also each cell layer were nuclear chimaeras, containing nuclei of two distinct origins and chromosome complements, within cytoplasm of one origin.

Sizes of adjacent haploid and diploid cells were markedly different. Incidentally, several tripolar spindles and aneuploid nuclei were also observed.

It follows that at the definite neural plate stage, the nuclei of the mesoderm and the neural plate itself are all capable of organizing cells of any cell layer and are not differentiated at least in this respect. As all hosts failed to complete development, it cannot be decided whether these nuclei differ in their capacity for further tissue differentiation.

In another series, ectodermal nuclei from the neural plate of diploid hosts were transplanted into haploid donors. This series is of less interest than the first one because the nuclei of haploid hosts might be expected in some cases to become diploid and thus be confused with donor nuclei; furthermore, haploid embryos are expected to be more prone to abnormalities. Nine hosts were fixed in this series, 7 as gastrulae and 2 as blastulae. In 3 no reliable chromosome counts could be made. Again, haploid and diploid cells were found in all cell layers of the other four.

As already indicated, the recipients that were not fixed at the early neural plate stage developed abnormalities and cytolysed soon afterwards. It is hoped however that these abnormalities might be avoided or delayed in further tests by: (a) implanting one donor nucleus only instead of a cluster of 2 to 4, and (b) grafting tissues from the chimaeras onto healthy normal embryos, where these tissues might differentiate further.

We wish to thank Professor C. H. WADDINGTON for his interest in this work and Dr. G. G. SELMAN for producing the haploid embryos used. We are thankful to the Melville Trust (E.M.P.) and the Royal Commission for the 1851 Exhibition (J.J.) for Research Fellowships.

E. M. PANTELOURIS and J. JACOB

Institute of Animal Genetics, Edinburgh, October 28, 1957.

Zusammenfassung

Gruppen von 2–4 somatischen Kernen haploider *Triturus*-Embryonen wurden in diploide, ungefurchte Eier injiziert. 11 haplo-diploide Mosaik-Larven entwickelten sich bis zum frühen Neurula-Stadium. Nachkommen von Kernen aus dem neuralen Ektoderm oder Chorda-Mesoderm von Spendern im Neurula-Stadium fanden sich in allen drei Keimblättern der Wirtslarve.

The Temporary Inactivation of Newt Larvae by Benzimidazole and its Alkyl Derivatives

Benzimidazole and some of its derivatives, produce muscle relaxation in mammals, apparently by their action on the central nervous system[1]. The experiments

[1] T. J. KING and R. BRIGGS, Cold Spring Harb. Symp. quant. Biol. *21*, 271 (1956).

[2] H. E. LEHMAN, Biol. Bull. *108*, 138 (1955). – C. H. WADDINGTON and E. M. PANTELOURIS, Nature *172*, 1050 (1953).

[1] L. GOODMAN, A. GILMAN, and N. HART, Fed. Proc. *2*, 80 (1943). – L. GOODMAN and N. HART, Fed. Proc. *3*, 73 (1944). – E. G. DOMINO, K. R. UNNA, and J. KERWIN, J. Pharmacol. *105*, 486 (1952).