The fact that there is practically no difference in the lipid values and their ratios in the young and old age group seems to be significant. This finding is in contradiction to some earlier views and speculations.[16] It compares favorably, however, with the finding that the plasma cholesterol and cholesterol ester values of infants are practically the same as those of adults.[17]

This constancy of the lipid values in different age groups indicates that even small changes, if uniformly encountered under pathological conditions, might prove to be significant.

Summary. 1. Total cholesterol, cholesterol esters, and phospholipid phosphorus were determined simultaneously in blood cells and plasma on 20 young healthy adults and 20 old patients with no known disorder of lipid metabolism. 2. Practically no difference was found in the lipid values of the 2 age groups. 3. Various ratios have been calculated from the lipid values determined. 4. The possible importance of similar studies under pathological conditions is pointed out.

16 Parhon, C. J., and Parhon, M., *Compt. Rend. soc. biol.*, 1923, **88**, 231.

17 Hodges, R. G., Sperry, W. M., and Andersen, O. H., *Am. J. Dis. Child.*, 1943, **65**, 858.

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Distribution of Cholesterol, Cholesterol Esters and Phospholipid Phosphorus in Blood in Thyroid Disease.

FRANCIS F. FOLDES AND ANNA J. MURPHY. (Introduced by L. Dienes).

From the Anesthesia Laboratory, Harvard Medical School, and the Thyroid Clinic of the Massachusetts General Hospital, Boston, Mass.

That changes of the plasma cholesterol level accompany thyroid diseases has been reported repeatedly.[1,2] The variations of the phospholipid phosphorus values have been investigated less frequently.[3,4] The most extensive recent study on this subject is that of Peters *et al.*[2] With the exception of a brief comment by Boyd,[5] no data have been published on the changes of the blood cell lipids in thyroid disease.

In a previous paper the distribution of some lipid values in blood cells and plasma of normal persons was presented.[6] In the following, the results of similar studies made on hypo- and hyperthyroid patients before and after adequate clinical treatment are reported, and an effort is made to correlate our findings with a new concept of the pathological chemistry of thyroid disease.

Material and Methods of Analysis. The studies were carried out on unselected patients of the Thyroid Clinic of the Massachusetts General Hospital. The only discrimination made was that mild cases of hyperthyroidism (basal metabolic rate below +30%) were excluded.

The hypothyroid group consisted of 7 patients, 6 of them female, one male. The ages of the patients ranged from 41 to 69 years. The basal metabolic rate was between +3% and —40% before, and between +11% and —13% after treatment. None of the patients received any form of thyroid medication within 6 months of the study.

1 Hurxthal, L. M., *Arch. Int. Med.*, 1933, **51**, 22; 1933, **52**, 86; 1934, **53**, 762.

2 Peters, J. P., and Man, E. B., *J. Clin. Invest.*, 1943, **22**, 715.

3 Gildea, E. F., Man, E. B., and Peters, J. P., *J. Clin. Invest.*, 1939, **18**, 739; Man, E. B., Gildea, E. F., and Peters, J. P., *J. Clin. Invest.*, 1940, **19**, 43.

4 Boyd, E. M., and Connell, W. F., *Quart. J. Med.*, 1939, **8**, 41.

5 Boyd, E. M., and Connell, W. F., *Quart. J. Med.*, 1936, **5**, 455.

6 Foldes, F. F., and Murphy, A. J., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 215.

Blood was taken for examination before, several times in the course of, and after adequate clinical treatment. The thyroid hormone was administered either in the form of thyreoglobulin or thyroid extract.

The hyperthyroid group comprised 12 patients, only 10 of whom were studied after treatment. Four of the patients were males and 8 females. The basal metabolic rates ranged from +52% to +33% before, and from +18% to —22% after treatment. Eight of the 10 cases followed received surgical treatment, and 2 were treated with radioactive iodine. Six of the surgical cases were prepared for operation with thiouracil, and 2 with potassium iodide. Lipids were determined before, in the course of, and after treatment. To save space, only the values determined before and after treatment will be presented in both the hypo- and the hyperthyroid group. The methods of analysis have been described.[6]

Results. The results of the analyses of the cell plasma lipid values are presented in Table I, where it can be seen that, with the exception of the cell total cholesterol and the cell phospholipid phosphorus, the lipid values of the untreated hypothyroid group were significantly increased. The increase of plasma total cholesterol was greater (over 100%) than that of plasma phospholipid phosphorus (less than 60%) and the relative changes of the cholesterol esters of cells and plasma were even more pronounced. After treatment the lipid values returned nearly to normal with the exception that the cell cholesterol esters became somewhat lower and the plasma cholesterol esters remained somewhat higher than the comparable values of the normal controls.

Similarly marked differences were found in all but one of the lipid ratios of untreated hypothyroid patients (Table II). The only ratio which showed no significant difference was the cell total cholesterol/cell phospholipid phosphorus ratio. The cholesterol ester/total cholesterol ratio and the total cholesterol/phospholipid phosphorus ratios in plasma were found to be increased, the cell cholesterol/plasma cholesterol and the cell phospholipid phosphorus/plasma phospholipid

TABLE I.

Mean Values and Standard Deviations of the Different Lipid Factors in Hypo- and Hyperthyroid Patients Before and After Treatment. Figures Marked with Asterisk Are Statistically Significant as Compared to Normal Values.

Group	No. of cases	Total cholesterol		Cholesterol esters		Phospholipid P	
		Cells, mg %	Plasma, mg %	Cells, mg %	Plasma, mg %	Cells, mg %	Plasma, mg %
Hypothyroid patients	Before treatment 7	198.8 ± 44.4	414.1 ± 79.1*	88.4 ± 49.0*	296.0 ± 43.5*	14.6 ± 1.5	14.3 ± 1.7*
	After treatment 7	189.3 ± 27.7	213.8 ± 32.1	3.1 ± 7.7*	152.0 ± 15.1*	13.7 ± 2.7	8.7 ± 1.8
Hyperthyroid patients	Before treatment 12	172.6 ± 31.0	156.2 ± 78.0	10.0 ± 16.5	98.2 ± 38.0*	14.5 ± 2.0	7.5 ± 2.1*
	After treatment 10	184.8 ± 50.7	242.2 ± 50.9*	10.0 ± 14.0	162.5 ± 40.6*	15.1 ± 1.0*	10.3 ± 2.2
Normal	40	173.0 ± 27.6	192 ± 35.5	15.3 ± 20.3	129.3 ± 25.0	14.1 ± 1.4	9.0 ± 1.2

TABLE II.
Mean Values and Standard Deviations of the Different Lipid Ratios in Hypo- and Hyperthyroid Patients Before and After Treatment. Figures Marked with Asterisk are Statistically Significant as Compared to Normal Values.

Group		No.	Cholesterol esters: Total cholesterol in plasma	Cell cholesterol: Plasma cholesterol	Cell phospholipid P: Plasma phospholipid P	Plasma cholesterol: Plasma phospholipid P	Cell cholesterol: Cell phospholipid P
Hypothyroid patients	Before treatment	7	0.73 ± 0.06*	0.50 ± 0.22*	1.04 ± 0.13*	28.68 ± 1.51*	13.62 ± 3.08
	After treatment	7	0.72 ± 0.06	0.93 ± 0.30	1.65 ± 0.77	24.86 ± 2.22*	13.44 ± 1.51
Hyperthyroid patients	Before treatment	12	0.62 ± 0.33	1.26 ± 0.58	2.05 ± 0.49*	20.96 ± 4.00	12.18 ± 1.90
	After treatment	10	0.69 ± 0.05	0.78 ± 0.21	1.52 ± 0.39	23.65 ± 4.20*	12.25 ± 2.60
Normals		40	0.68 ± 0.06	0.94 ± 0.26	1.60 ± 0.29	21.30 ± 2.66	12.30 ± 2.24

phosphorus ratios were found to be decreased, as compared to normals. After treatment all ratios changed towards normal. Only the total cholesterol/phospholipid phosphorus ratio of the plasma remained somewhat elevated.

The changes found in the mean values of the different lipid factors of the untreated hyperthyroid group are less marked, and the standard deviations are relatively greater than in the untreated hypothyroid group (Table I). Consequently, statistically significant differences can only be found in the values of the plasma cholesterol esters and the plasma phospholipid phosphorus. Following treatment of the hyperthyroid patients all lipid values increased and with the exception of the cell cholesterol esters their mean values became higher than the corresponding values of the normal control group. The increase of the plasma cholesterol, plasma cholesterol ester, and cell phospholipid phosphorus was great enough to be statistically significant.

The situation is much the same with regard to the different lipid ratios (Table II). Before treatment, the changes are opposite in direction to those found in the hypothyroid group. But owing to the fact that the differences of the mean values are less, and the coefficients of variation are greater, only the cell phospholipid phosphorus/plasma phospholipid phosphorus ratio differs significantly from the normal. After treatment the ratios change toward normal and in some instances (cell cholesterol/plasma cholesterol, cell phospholipid phosphorus plasma/phospholipid phosphorus, plasma cholesterol/plasma phospholipid phosphorus ratios) even overshoot in the direction of the hypothyroid values.

Discussion. Recently Hoffmann and Hoffmann[7] in a stimulating monograph advanced an entirely new concept of the pathological chemistry of hyperthyroidism, with which they present considerable evidence both from their own experiments and from the results of other workers. Since our clinical data seem to be in agreement with some of their

[7] Hoffmann, F., and Hoffmann, E. J. de, *Public. del instit. fisiol.*, Universidad de Chile, 1943.

views, we should like to summarize, in the following paragraph, the main points of their theory before discussing our findings.

They believe that due to the action of certain enzymes (lecithinases), continuous breakdown of phospholipids occurs in the organism. One of these breakdown products, lysolecithin,[8] has marked biological activity. It plays an important role in the metabolism of the central nervous system; it influences cell permeability for certain substances, and if present in increased concentration, it exerts toxic effects on various organs. These breakdown products can be demonstrated in the blood in increased amounts after stimulation of the cervical sympathetic trunk, the vagus, or sciatic nerve. Under normal conditions the toxic effects of lysolecithin are neutralized by various sterols, mainly cholesterol and the steroid hormones of the adrenals, perhaps also of the gonads. The thyroid hormone has a stimulating effect on lysolecithin formation. Consequently, in hyperthyroidism, where an excess of thyroid hormone is produced, the lysolecithin formation is also increased. The organism tries to counteract the toxic effect of lysolecithin by mobilizing its sterol reserves. As long as these reserves are ample the toxic manifestations of hyperthyroidism are kept under control, but when the sterol reserves have been exhausted the toxic symptoms become manifest.

The changes of the blood lipids that other workers, as well as ourselves, have found in thyroid diseases are at least not at variance with these assumptions. Table I shows that in hypothyroidism both the plasma cholesterol and plasma phospholipid phosphorus are increased. The relative increase of the plasma cholesterol is greater than that of the plasma phospholipid phosphorus. This finding, as well as the marked increase of the plasma cholesterol/phospholipid phosphorus ratio are in agreement with the findings of Peters *et al.*[2] If the thesis that the decomposition of lecithin is regulated by the thyroid hormone is accepted, it seems logical that with decreased thyroid activity the breakdown of lecithin should be curtailed and the plasma phospholipid phosphorus level should rise. Moreover, since the available sterols (especially cholesterol) are not used up for the neutralization of lysolecithin, the accumulation of cholesterol in plasma can also be considered as satisfactorily explained.

After adequate treatment with thyroid hormone, conditions change in the direction of normal. Under the stimulating effect of the hormone the decomposition of phospholipids returns to normal, cholesterol is used up in increased amounts to neutralize the lysolecithin formed, and consequently both the cholesterol and phospholipid levels become lower.

Similar considerations might satisfactorily explain the chain of events in hyperthyroidism. In this condition the increased amount of thyroid hormone might cause increased phospholipid breakdown and result in increased lysolecithin formation. The organism tries to neutralize the toxic lysolecithin by mobilizing its sterol reserves and probably also by increasing the synthesis of cholesterol and steroid hormones. It can be assumed that as long as this compensatory mechanism is able to keep pace with the increased lysolecithin formation the patients are more or less compensated from the clinical point of view. The exhaustion of this compensating mechanism results in the rapid deterioration of the patient's condition and in extreme cases it might be manifested in the form of thyroid crisis. In agreement with this assumption the most constant change encountered in the blood lipid values of hyperthyroidism was the decrease of the plasma phospholipid phosphorus. The average plasma cholesterol level of 12 cases was also decreased, but as the relatively high coefficient of variation indicates, this decrease was not distributed evenly over all the cases. The same was found to be true for the plasma cholesterol/phospholipid phosphorus ratio. Following clinical treatment the lipid values not only return to normal, but even overshoot in the direction of the hypothyroid levels. The reason for this is undoubtedly the fact that, in contrast to hypothyroid pa-

[8] Feldberg, W., *Ann. Rev. Physiol.*, 1941, **3**, 671.

tients, hyperthyroid patients have to be overtreated in order to obtain best clinical results. That this is so is indicated by the fact that after adequate surgery, (especially after thiouracil preparation), the basal metabolic rate is usually lower than normal and values of —15% to —20% are not rare.

The blood lipid changes became normal under iodine or thiouracil preparation even before surgery. The same was found to be true in 2 patients who were medically treated with radioactive iodine.

The hitherto unexplained beneficial effect of KI in hyperthyroidism might perhaps also be explained by a primary effect on lipids. It was found that KI administration caused a further elevation of the plasma cholesterol level in hypercholestermic rabbits.[9,10]

Both histological[11-14] and functional studies[15-17] have established the existence of liver involvement in hyperthyroidism. It is also generally accepted[18] that in liver diseases the plasma ester cholesterol/total cholesterol ratio is decreased. In agreement with these facts the average plasma cholesterol ester/total cholesterol ratio in our 12 cases was found to be 0.62 ± 0.33 before treatment as compared to the normal average of 0.68 ± 0.06. As the large coefficient of variation indicates, the variation of the individual ratios, just as in the case of the other lipids values in hyperthyroidism, was very large.* It has been shown by some workers that in general the plasma cholesterol level is inversely proportional to the clinical toxicity of hyperthyroid patients and the plasma cholesterol level increases after treatment.[2,19] We have now found that in our small series the plasma ester cholesterol/total cholesterol ratio behaves similarly: the more toxic the patient, the lower the ratio. Following medical or surgical treatment, not only the ratio but also its coefficient of variation returns to normal.

We should like to emphasize that the Hoffmann hypothesis can by no means be considered as proven. On the other hand, there is enough suggestive evidence in its support to warrant further clinical and pharmacological studies. An example of its possible usefulness is that the hitherto unexplained blood lipid changes of thyroid disease can be interpreted by it in a way which is in agreement with the already known antagonistic properties of cholesterol and lecithin.[20]

Summary. 1. The distribution of total cholesterol, cholesterol esters, and phospholipid phosphorus was studied in the blood cells and plasma of thyroid patients. 2. The plasma cholesterol, plasma cholesterol ester, and plasma phospholipid phosphorus were found to be significantly increased in hypothyroid patients. 3. The changes of the plasma lipids were less consistent in hyperthyroidism. The plasma phospholipid phosphorus alone showed a significant decrease. 4. The cell lipid values were found relatively constant both in hypo- and hyperthyroidism. 5. Definite changes were also found in the different lipid ratios in hypothyroidism. The plasma cholesterol ester/total cholesterol ratio and the plasma cholesterol/plasma phospholipid phosphorus ratio were significantly increased. The cell cholesterol/plasma cholesterol and the cell phospholipid phosphorus/plasma phospholipid phosphorus ratios were significantly decreased. Only the cell cholesterol/cell phospholipid phosphorus

[9] Rosenthal, S. R., *Arch. Path.*, 1934, **18**, 827.

[10] Page, I. H., and Bernhard, W. G., *Arch. Path.*, 1935, **19**, 530.

[11] Bert, J. E., and Goldburgh, H. L., *Internat. Clinic.*, 1941, **4**, 126.

[12] Beaver, D. C., and Pemberton, J. DeJ., *Ann. Int. Med.*, 1933, **7**, 687.

[13] Weller, C. V., *Ann. Int. Med.*, 1933, **7**, 543.

[14] Lord, J. W., and Andrus, W. deW., *Arch. Surg.*, 1941, **42**, 643.

[15] Haines, S. F., Magath, T. B., and Power, M. H., *Ann. Int. Med.*, 1941, **14**, 1225.

[16] Smith, M. C., Johndahl, W., and Ochsner, A., *Surg. Gyn. Obs.*, 1942, **74**, 1083.

[17] Mills, F. H., *Med. J. of Australia*, 1942, **1**, 195.

[18] Man, E. B., Kartin, B. L., Durlacher, S. H., and Peters, J. P., *J. Clin. Invest.*, 1945, **24**, 623.

* In contrast to our findings, Peters *et al.* found no changes in the plasma cholesterol partition of 9 thyroid patients.

[19] Cutting, W. C., Rytand, O. A., and Tainter, L. M., *J. Clin. Invest.*, 1934, **13**, 574.

[20] Degkwitz, R., *Ergeb. Physiol.*, 1931, **32**, 821.

ratio remained unchanged. 6. In hyperthyroidism the change in the ratios was again less constant. The only significant change was the increase of the cell phospholipid phosphorus/plasma phospholipid phosphorus ratio. 7. Both the lipid values and the various lipid ratios returned towards normal following adequate treatment.

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Relation Between Metabolic Activity and Cyanide Inhibition in *Pelomyxa carolinensis* Wilson.*

D. M. PACE AND T. E. KIMURA.

From the Department of Physiology and Pharmacology, College of Pharmacy, University of Nebraska.

Many investigations have been made on various animal species in ascertaining the presence or absence of a cytochrome-cytochrome oxidase system in living cells. In recent years numerous reports have been published in which protozoan cells have been used as experimental organisms. Some of these unicellular forms make excellent material for this type of investigation, and all of them are sensitive to cyanides, although, formerly, there was some doubt about this, especially in reference to ciliates.

Lund,[1] Shoup and Boykin,[2] and Gerard and Hyman[3] maintained that *Paramecium caudatum* was non-sensitive to HCN. Peters[4] claimed that respiration in *Colpidium colpoda* was not inhibited by potassium cyanide and Pitts[5] working with *Colpidium campylum* and Lwoff[6] with *Glaucoma piriformis* found that there was an initial inhibition when these forms were exposed to cyanide, but that oxygen consumption soon increased to normal. Hall,[7] however, definitely proved that cyanide inhibits respiration in *Colpidium campylum* and Baker and Baumberger[8] found the same to be true for *Tetrahymena geleii.*

The apparent non-sensitivity of *Paramecium* to cyanide was questioned by Hyman in unpublished data referred to by Child[9] and Pace.[10] Saito and Tamiya[11] found cytochrome in *Paramecium,* which led to the supposition that respiration in *Paramecium* might depend, at least partly, upon cytochrome-cytochrome oxidase. Since that time, Boell[12] working with *Paramecium calkinsi,* Pace[10] with *P. caudatum* and *P. aurelia;* and Clark[13] with *P. caudatum* found that oxygen consumption of these species is inhibited by cyanide. Evidence has now accumulated to such an extent that the sensitivity of ciliates to cyanide is no longer questioned.

According to Pace,[10] sensitivity to cyanide in *P. aurelia* and *P. caudatum* is apparently dependent upon the degree of carbohydrate metabolism. He finds that inhibition is much more pronounced when glucose is pres-

* These investigations were partly supported by a grant-in-aid obtained from the Society of Sigma Xi.

1 Lund, E. J., *Am. J. Physiol.,* 1918, **45**, 365.

2 Shoup, C. S., and Boykin, J. T., *J. Gen. Physiol.,* 1931, **15**, 107.

3 Gerard, R. W., and Hyman, L. H., *Am. J. Physiol.,* 1931, **97**, 524.

4 Peters, R. A., *J. Physiol.,* 1929, **68**, 11.

5 Pitts, R. F., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 542.

6 Lwoff, M., *C. R. Soc. Biol.,* Paris, 1934, **115**, 237.

7 Hall, R. H., *Physiol. Zool.,* 1941, **14**, 193.

8 Baker, E. G. S., and Baumberger, J. P., *J. Cell. Comp. Physiol.,* 1941, **17**, 285.

9 Child, C. M., *Patterns and Problems of Development,* 1941, 811.

10 Pace, D. M., *Biol. Bull.,* 1945, **89**, 76.

11 Saito, T., and Tamiya, H., *Cytologia,* 1937, Fujii Jubilee Volume, 1133.

12 Boell, E. J., *Anat. Rec.,* 1942, **84**, 493.

13 Clark, A. M., *Aust. J. Exp. Biol. and Med.,* 1945, **23**, 317.