THE INFLUENCE OF THYROIDECTOMY, SPLENECTOMY, GONADECTOMY, AND SUPRARENALECTOMY UPON THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS IN RABBITS.*

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(Received for publication, November 30, 1926.)

Ignatowski (1) showed in 1908 that in rabbits the prolonged feeding of foods of animal origin caused atherosclerosis of the aorta. He believed that the proteins were responsible for the intimal changes. It was subsequently found by Stuckey (2) and by Wesselkin (3) that only cholesterol-containing substances produced these arterial changes and it was finally determined that cholesterol was the essential constituent when Anitschkow and Chalatow (4) and Wacker and Hueck (5) produced atheromata of the aorta by feeding pure cholesterol in oil to rabbits. Later Aschoff and his coworkers (6) demonstrated that the anisotropic crystals deposited within the areas of intimal swelling and softening were cholesterol esters.

The similarity of experimental atheromata to those seen in humans was pointed out by various authors, especially Anitschkow (7), Aschoff (8), Zinserling (9), Klotz (10), and Bailey (11). It was recognized, as has been emphasized by Bailey (11), that although the experimental atheromata are practically identical with those seen in man, constantly associated with the artificially produced lesions are fatty deposits in other tissues such as spleen, liver, and kidneys not usually accompanying atherosclerosis in man. Anitschkow (12) therefore fed to rabbits over long periods of time (about 2 years) diets containing small doses of cholesterol, producing only very slight or no hypercholesterolemia. At autopsy these animals showed atheromata but no deposits in the other organs.

* Presented in abstract before the American Society for Experimental Pathology, Cleveland, December 28, 1925.

Much additional work on experimental atherosclerosis has been done and numerous reports published from various sources, especially Russia, Germany, and this country. Historical reviews have been included in several of the older and more recent papers. Therefore, no attempt will be made at this time to cover this phase of the subject and those interested are referred to the publications of Rickett (13), Dewey (14), Schönheimer (15), Bailey (11), and Chuma (16).

Comprehensive descriptions of the intimal lesions as they occur in man and in rabbits following cholesterol feedings have been published by various authors, especially those already mentioned, and are so well known that we shall merely refer to them briefly. The lesions observed by us were identical with those reported by these workers and may be described as follows:

Blocks were taken from the thoracic aorta, usually at the arch, and fixed in 10 per cent formalin. Celloidin sections were stained with hematoxylin and eosin, frozen sections with Sudan III and hematoxylin, and unstained sections, mounted in glycerol jelly, were examined with the polarizing microscope. In some instances paraffin sections were stained with Weigert's elastic tissue stain and counterstained with Van Gieson's stain.

Because this was a comparative study we classified the lesions into (1) early atheromatosis, (2) moderate, and (3) severe atheromatosis.

1. Early Atheromatosis.—These were the earliest changes observed. There were sparsely distributed swellings of the vessel wall which macroscopically appeared as punctate elevations on the intima. Microscopically they consisted of local thickenings of the intima in which were present large irregularly shaped cells occurring in one or several layers and containing a substance which stained with Sudan III, a small amount of which was anisotropic. The internal elastic membrane and the outer layers of the aortic wall showed no alterations at this stage.

2. Moderate Atheromatosis.—Macroscopically the lesions were more numerous and larger. Microscopically at this stage there was further thickening of the intima with an increase in the number of large irregular "foamy" cells. The internal elastic membrane was more prominent and showed some separation of its fibers. Within the media there was a small amount of fat-staining material which was anisotropic.

3. Severe Atheromatosis.—In severe atheromatosis the lesions extended over the greater part of the intimal surface of the aorta. Microscopically the intima was much more thickened and the large foamy cells were still more numerous. Fibroblasts were visible. The Sudan-staining anisotropic material was more abundant both in the intima and media. There was much more separation of the elastic fibers at this stage.

More recent work by Murata and Kataoka (17) and by Chuma (16) has shown that castration tends to facilitate the development of atheromata after lanolin (cholesterol) feeding. Murata (18) reported further that thyroid administration to these rabbits inhibits atheroma formation.

Because of their susceptibility to the development of atherosclerosis rabbits have been used in these experiments. It has been possible also to produce the same changes in guinea pigs (Anitschkow and Chalatow (4), Schönheimer (15)) and in omnivorous animals, especially after castration (Löwenthal (19)).

The possible occurrence of spontaneous atheromata in rabbits is of the greatest importance in this work. There are many factors which may be responsible for the development of the condition, notably, age of the animal, hygienic conditions, previous infections, and diet. The frequency of spontaneous atherosclerosis has, therefore, been observed and commented upon by various authors. The findings, however, have been in wide disagreement. For example, Miles (20) found 17 out of 49 rabbits to have spontaneous atheromata while Steinbiss (21) could find none in 500 rabbits examined. Accordingly van Leersum (22) in 1914 tabulated the findings of various authors up to that time. Of 1937 rabbits examined spontaneous lesions were found in 49 instances. In addition to these Ophüls (23) has reported none present in 50 rabbits while Weinberg (24) found up to 9 per cent of 692 rabbits to have spontaneous atheromata.

Therefore, it is of the greatest significance that all the rabbits used in the experiments reported in this paper were of known age, were born and reared in this laboratory, lived under practically identical conditions since birth, and were apparently healthy and vigorous when selected for the experiments. Furthermore, it is noteworthy that of more than 250 rabbits which came to autopsy in this laboratory, a number of them litter mates of the rabbits used in these experiments, and which were examined for atheromata, only 1 had spontaneous lesions in the aorta. This rabbit had long standing snuffles and showed extensive chronic pulmonary disease at autopsy.

Cholesterol has been administered in various forms for the production of hypercholesterolemia and atherosclerosis. It has been given in pure form by intravenous injection in sesame oil (Adler (25)),

emulsified with sodium oleate (Klotz (10)), intraperitoneally in aqueous emulsion (Dewey (14)), and by feeding cholesterol-containing substances such as egg yolk, brain substance, dried liver (Anitschkow (7), Stuckey (2), Steinbiss (21), Bailey (11), and others). Lanolin (adeps lanæ hyd.) first used by Kon (26), contains cholesterol in large quantities and has been found to be an excellent means for introducing this substance into the body by feeding.

In a previous series of experiments (unpublished) we used the aqueous emulsion described by Dewey (14) intravenously and intraperitoneally but with unsatisfactory results. In the experiments herein reported we fed the lanolin in oil in addition to the regular laboratory diet of alfalfa hay, oats, and vegetables. The dose of lanolin used by us was 4 gm. daily in 12 cc. cottonseed oil. The mixture was heated to about body temperature and fed by pipette.

As has already been pointed out it has been found that cholesterol plays an essential rôle in the formation of experimental atheromata of the aorta. We have, therefore, studied some of the factors which are believed to influence the metabolism of this fatty substance and upon which might depend its abnormal deposition within the intima of the aorta. It is the purpose of this paper to present evidence to show the influence of feeding lanolin in oil on the production of experimental atherosclerosis in (1) normal rabbits, (2) thyroidectomized rabbits, (3) gonadectomized rabbits, (4) splenectomized rabbits, and (5) suprarenalectomized rabbits.[1]

In this report are included only the 44 animals which lived over 25 days. We excluded from the series 10 rabbits which died of marasmus due to toxicity of cottonseed oil and 4 which died of acute suprarenal insufficiency.

1. Normal Rabbits.—We found that rabbits with all organs intact sacrificed after 110 days of feeding with lanolin in oil showed early atheromata in the aorta. 3 rabbits sacrificed after 90, 95, and 100 days of the feeding respectively showed no atheromata. The others, killed after 110 days or longer, all showed atheromata.

2. Gonadectomized Rabbits.—Confirming Kon, Murata, and Chuma, we observed that gonadectomized rabbits developed atheromata more

[1] All operations were performed under ether anesthesia.

readily than those with all organs intact. After 50 to 60 days of feeding with lanolin the castrates showed early atheromata. There were 2 exceptions: 1 sacrificed after 50 and the other after 90 days showed no atheromata.

TABLE I.

Number of rabbits in group	Operative procedure	Period of lanolin feeding	Degree of atheromatosis	Remarks
		days		
10	None	110–120	Early	
6	Gonadectomy	50–60	Early	Two fed 50 and 90 days showed no atheromata
7	Splenectomy	50–60	Early and moderate	
8	Thyroidectomy	40–50 70	Moderate Severe	
6	Double suprarenalectomy	85–100 110	None Early	Largest thyroids 8 to 10 times normal size. Lymphoid overgrowth
1	Thyroidectomy and double suprarenalectomy	50	None	
1	Thyroidectomy and splenectomy	50	Severe	
3	Double suprarenalectomy and splenectomy	45 106–110	None Early	
2	Gonadectomy and splenectomy	100	Severe	

3. Splenectomized Rabbits.—After 50 to 60 days of feeding with lanolin in oil the splenectomized rabbits showed early and moderate atherosclerosis. The lesions were more extensive than those seen in the castrated animals which were fed over equal periods of time.

4. Thyroidectomized Rabbits.—Thyroid-deficient rabbits showed

moderate atheromata after 40 to 50 days and severe atheromata after 70 days of feeding with lanolin. This group was the most susceptible.

5. Suprarenalectomized Rabbits.—10 doubly suprarenalectomized rabbits were used. Of these 1 was also thyroidectomized and 3 splenectomized. Of the first 6, 5 were fed between 85 and 100 days. None of these showed atheromata. The remaining one was sacrificed after 110 days of feeding. Early atheromata were present. The thyroidectomized and suprarenalectomized rabbit was fed 50 days. The aorta was free from atheromata at autopsy. Of the 3 splenectomized and suprarenalectomized, 1 fed 45 days on lanolin showed early atheromata.

At the same time, for controls, 1 thyroidectomized and splenectomized rabbit was fed 50 days and 2 gonadectomized and splenectomized rabbits were fed 100 days. At autopsy all 3 showed severe atheromata of the aorta.

DISCUSSION.

We find, as have others, that deposition of cholesterol or cholesterol esters within the intima of the aorta initiates the cellular alterations which result in the formation of atheromata. We have readily produced it experimentally in rabbits by inducing prolonged alimentary hypercholesterolemia. We find further that thyroidectomy, splenectomy, and gonadectomy each facilitate and accelerate the development of this condition within the aorta. The thyroid-deficient rabbits are most susceptible and the splenectomized group are slightly more susceptible than the castrates.

The question arises: Why does removal of the gonads, the spleen, or the thyroid facilitate the development of experimental atheromata? In the case of the spleen we may be eliminating a possible storehouse for excess cholesterol because it sometimes undergoes very marked enlargement in the presence of prolonged lipemia. This is well illustrated in experimental lipemia and clinically in Gaucher's disease and in some cases of diabetic lipemia (1, 27, 5, 15, 28, 29, 30, and others). The relation of the spleen to the reticulo-endothelial system and its function in both the destruction of old red blood cells and the formation of new erythrocytes emphasizes the possibility that the spleen not only stores cholesterol but also utilizes it. Thus also it has been

found that splenectomy alone is followed by hypercholesterolemia (31–39).

Concerning the relation of the gonads to cholesterol metabolism very little is known. Their removal, however, may give rise to a slight hypercholesterolemia (40, 41). Certainly they do not store the fatty substance in any appreciable amount although the interstitial cells may appear more prominent in prolonged hypercholesterolemia. To what extent cholesterol is utilized in ovarian or testicular function still remains to be demonstrated. Clinically, menopause cases are said to show a tendency to develop hypercholesterolemia (42). One other possibility deserves emphasis, however, namely, the interrelation of the sex glands with the thyroid. It appears that a synergistic functional relationship operates between the gonads and the thyroid. For example, gonadectomy may cause a slight drop in heat production and even involution of the thyroid (43–49). Also, it is known that at those periods when the sex glands are the seat of both physiological and morphological alterations (puberty, pregnancy, climacteric) the thyroid may likewise undergo striking changes, sometimes to pathological extents so that exophthalmic goiter, simple goiter, or myxedema (Gull's disease) may result. In all probability, therefore, the importance of the gonads in cholesterol metabolism is dependent upon the interrelationship which exists between the sex glands and the thyroid (46, 50).

The thyroid on the other hand plays what appears to be an essential rôle in the metabolism of the fats and fat-like substances, including cholesterol. Exactly what processes in the burning of fats are dependent upon the presence of thyroxin for their completion cannot be stated at the present time. There is good evidence, however, that high fat diets cause increased thyroid activity. Indeed, unless iodine is administered to prevent it, thyroid overgrowth soon results. This has been amply illustrated in the work of Marine and Lenhart (51), McCarrison (52), the Mellanbys (53), and ourselves (54). We have observed further that such thyroid insufficiency produced by feeding neutral fat (cottonseed oil) over long periods of time is accompanied (after 60 days or longer) by hypercholesterolemia and that this increase in the cholesterol content of the blood is directly proportional to the degree of thyroid insufficiency (*i.e.* hyperplasia) (55).

We find that thyroidectomy in rabbits facilitates and accelerates the deposition of cholesterol and the development of experimental atheromata. The reason appears to be that thyroid-deficient animals are less able to oxidize fats and fatty substances (cholesterol) than normals. Marked hypercholesterolemia, cholesterol deposition, and, in turn, consequent atheroma formation result. In direct accord with this is the old and well known clinical observation that thyroid-deficient animals and man are very prone to develop hypercholesterolemia and atheromatosis (56–61, and others).

At this juncture it should be pointed out that since iodine is indispensable for thyroid function the beneficial effects known to follow the prolonged administration of iodides clinically in atheromatosis and atherosclerosis may be dependent upon the influence that such iodine compounds have upon thyroid activity. There is some evidence that the cholesterol content of the blood can be lowered by the administration of thyroid or iodine (62–64). Marine has demonstrated clearly the effects of small (physiological?) doses of iodine upon thyroid hyperplasia. It seems, however, that large or massive doses of iodine may give rise to additional and important reactions. This is the subject of some investigations now being conducted.

Our evidence indicates that sublethal suprarenal insufficiency does not aid in the deposition of cholesterol and the development of experimental atheromata.

The suprarenalectomized rabbits which we used had recovered from the acute insufficiency following the removal of at least one and three-fourths glands and were well compensated during the subsequent experimental period. That they suffered from varying degrees of suprarenal insufficiency was indicated by the fact that they showed at autopsy some lymphoid overgrowth involving both thymus and lymph nodes and very large hyperplastic thyroids, in 3 instances 8 to 10 times the average normal size. Complete double suprarenalectomy was not done in all cases. A fragment of one gland was left behind purposely in 4 of these animals. Thus, we found that rabbits in a state of sublethal suprarenal insufficiency were not more susceptible to the development of atheromata of the aorta following the feeding of lanolin than rabbits with all organs intact. These findings confirm indirectly the observations of Baumann and Holly (65). In 4 in-

stances (1 thyroidectomized and 3 splenectomized) we obtained evidence which suggests that such a state of suprarenal insufficiency may exert an inhibitory influence upon the development of experimental atherosclerosis.

A propos of this it might be mentioned that atherosclerosis is rare in individuals with lymphatism, status thymicolymphaticus, and similar conditions characterized by lymphoid overgrowth. A constitutional anomaly of similar nature seems to result in animals in which a state of sublethal suprarenal insufficiency has been experimentally produced (66).

All this evidence indicates that conditions which are accompanied by long standing hypercholesterolemia cause or facilitate the deposition of cholesterol within the intima of the aorta. This, we find, as have numerous other workers, initiates the formation of experimental atheromata.

In these experiments with lanolin feeding there was no hypertension (67). The blood pressure readings were determined by the method described by Anderson (68). In other words, we find that experimental atheromatosis in rabbits may develop in the presence of normal blood pressure.

However, we do not maintain that prolonged hypertension when present may not alter the arterial wall so that atheromatosis results. The same applies to other mechanical influences such as forces of pulling and dragging, and of wear and tear, the effects of which are seen so frequently in man in later life (Aschoff (69)). Our findings indicate that the deposition of cholesterol within the intima of the aorta initiates the formation of atheromata and any condition (hypercholesterolemia, mechanical forces, etc.) which predisposes to such precipitation thereby predisposes to the development of atheromatosis. This is in agreement with the findings of Aschoff and of Wacker and Hueck.

SUMMARY.

Alimentary hypercholesterolemia acting over a sufficient period of time (in rabbits with all organs intact, 110 days or longer) causes deposition of cholesterol within the intima of the aorta. Deposition of cholesterol within the intima of the aorta initiates the formation of

experimental atheromata. Thyroidectomy, splenectomy, and gonadectomy augment hypercholesterolemia and thereby facilitate and accelerate the development of experimental atheromata of the aorta in rabbits. Sublethal suprarenal insufficiency does not increase the susceptibility of rabbits to the development of such atheromata.

The author wishes to express his gratitude to Dr. David Marine and to Dr. E. J. Baumann for their assistance and helpful advice.

BIBLIOGRAPHY.

1. Ignatowski, A., Über die Wirkung des tierischen Eiweisses auf die Aorta und die parenchymatösen Organe der Kaninchen, *Virchows Arch. path. Anat.*, 1909, cxcviii, 248.
2. Stuckey, N. W., Über die Veränderungen der Kaninchenaorta unter der Wirkung reichlicher tierischer Nahrung, *Zentr. Path.*, 1911, xxii, 379; Über die Veränderungen der Kaninchenaorta bei Fütterung mit verschiedenen Fettsorten, *Zentr. Path.*, 1912, xxiii, 910.
3. Wesselkin, N. W., Über die Ablagerung von fettartigen Stoffen in den Organen, *Virchows Arch. path. Anat.*, 1913, ccxii, 225.
4. Anitschkow, N., and Chalatow, S., Ueber experimentelle Cholesterinsteatose und ihre Bedeutung für die Entstehung einiger pathologischer Prozesse, *Centr. allg. Path. u. path. Anat.*, 1913, xxiv, 1.
5. Wacker, L., and Hueck, W., Ueber experimentelle Atherosklerose und Cholesterinämie, *Münch. med. Woch.*, 1913, lx, 2097.
6. Aschoff, L., Zur Morphologie der lipoiden Substanzen, *Beitr. path. Anat. u. allg. Path.*, 1910, xlvii, 1. Kawamura, R., Die Cholesterinesterverfettung (Cholesterinsteatose), Jena, 1911.
7. Anitschkow, N., Über die Atherosklerose der Aorta beim Kaninchen und über deren Entstehungsbedingungen, *Beitr. path. Anat. u. allg. Path.*, 1914, lix, 306.
8. Aschoff, L., Arteriosklerose, *Beihefte Med. Klin.*, 1914, x, 1.
9. Zinserling, W. D., Ueber anisotrope Verfettung der Aortenintima bei Infektionskrankheiten, *Centr. allg. Path. u. path. Anat.*, 1913, xxiv, 627.
10. Klotz, O., Fatty degeneration of the intima of arteries, *J. Med. Research*, 1915, xxxii, 27. McMeans, J. W., and Klotz, O., Superficial fatty streaks of arteries: an experimental study, *J. Med. Research*, 1916, xxxiv, 41.
11. Bailey, C. H., Atheroma and other lesions produced in rabbits by cholesterol feeding, *J. Exp. Med.*, 1916, xxiii, 69.
12. Anitschkow, N., Zur Ätiologie der Atherosklerose, *Virchows Arch. path. Anat.*, 1924, ccxlix, 73.
13. Rickett, G. R., Experimental atheroma, *J. Path. and Bact.*, 1908, xii, 15.

14. Dewey, K., Experimental hypercholesterolemia, *Arch. Int. Med.*, 1916, xvii, 757.
15. Schönheimer, R., Über die experimentelle Cholesterinkrankheit der Kaninchen, *Virchows Arch. path. Anat.*, 1924, ccxlix, 1.
16. Chuma, M., Über Organveränderungen nach Lanolinfütterung beim Kaninchen, *Virchows Arch. path. Anat.*, 1923, ccxlii, 275.
17. Murata, M., and Kataoka, S., Experimentelle Arteriosklerose und Schilddrüsenfütterung, *Verhandl. jap. path. Ges.*, 1918, viii, 221, quoted by Aschoff (69).
18. Murata, *Nisshin. Igaku*, vii, No. 8, quoted by Chuma.
19. Löwenthal, K., *Med. Klin.*, 1926, xxii, 770; abstracted in *J. Am. Med. Assn.*, 1926, lxxxvii, 210.
20. Miles, A. B., Spontaneous arterial degeneration in rabbits, *J. Am. Med. Assn.*, 1907, xlix, 1173.
21. Steinbiss, W., Über experimentelle alimentäre Atherosklerose, *Virchows Arch. path. Anat.*, 1913, ccxii, 152.
22. van Leersum, E. C., Zur Frage der experimentellen alimentären Atherosklerose, *Virchows Arch. path. Anat.*, 1914, ccxvii, 452.
23. Ophüls, W., Occurrence of spontaneous lesions in kidneys and livers of rabbits and guinea pigs, *Proc. Soc. Exp. Biol. and Med.*, 1910–11, viii, 75.
24. Weinberg, quoted by Rubinstein, M., L'athérome expérimental par ingestion de cholestérine, *Compt. rend. Soc. biol.*, 1917, lxxx, 191.
25. Adler, I., Further studies in experimental atherosclerosis, *J. Exp. Med.*, 1917, xxvi, 581.
26. Kon, Y., Adenoma-formation in the stomach of rabbits by feeding with lanolin, *J. Med. Research*, 1917, xxxv, 337.
27. Anitschkow, N., Über experimentell erzeugte Ablagerungen von anisotropen Lipoidsubstanzen in der Milz und im Knochenmark, *Beitr. path. Anat. u. allg. Path.*, 1914, lvii, 201.
28. Lutz, W., Über grosszellige Hyperplasie der Milzpulpa bei diabetischer Lipämie, *Beitr. path. Anat. u. allg. Path.*, 1914, lviii, 273.
29. Schultze, W. H., Ueber grosszellige Hyperplasie der Milz bei Lipoidaemie (Lipoidzellenhyperplasie), *Verhandl. deutsch. path. Ges.*, 1912, xv, 47.
30. Smith, M. G., Hyperplasia of lipoid-holding cells in diabetes with lipemia, *Bull. Johns Hopkins Hosp.*, 1925, xxxvi, 203.
31. Eppinger, H., Zur Pathologie der Milzfunktion, *Berl. klin. Woch.*, 1913, l, 1509, 1572.
32. Soper, W. B., Zur Physiologie des Cholesterinstoffwechsels. VI. Über Beziehungen der Milz zum Cholesterinstoffwechsel, *Beitr. path. Anat. u. allg. Path.*, 1915, lx, 232.
33. Siegmund, H., Untersuchungen über den Einfluss der Milzexstirpation auf dem Fettgehalt des Blutes, *Virchows Arch. path. Anat.*, 1917, ccxxiv, 303.
34. Medak, E., Beitrag zur Chemie des Blutes bei anämischen Krankheitsbildern, *Biochem. Z.*, 1914, lix, 419.

35. King, J. H., The effect of removal of the spleen upon the metabolism in dogs, *Johns Hopkins Hosp. Rep.*, 1919, xviii, 7.
36. Denis, W., The influence of splenectomy on metabolism in anemia, *Arch. Int. Med.*, 1917, xx, 79.
37. MacAdam, W., and Shiskin, C., The cholesterol content of the blood in anæmia, and its relation to splenic function, *Quart. J. Med.*, 1923, xvi, 193.
38. Ducuing, Rouzaud, J. J., and Soula, C., La cholesterinémie chez les splénectomisés, *Compt. rend. Soc. biol.*, 1924, xc, 263.
39. Campbell, J. M. H., Critical review. Cholesterol in health and disease, *Quart. J. Med.*, 1925, xviii, 393.
40. Luden, G., Observations on the changes in the cholesterol content of the blood of goats, following cholesterol feeding alone, Roentgen treatment alone, and cholesterol feeding combined with Roentgen treatment and subsequent castration, *J. Biol. Chem.*, 1916, xxvii, 273.
41. Löwenthal, K., Zur Physiologie des Cholesterinstoffwechsels. Beziehungen zwischen Hoden und Cholesterinstoffwechsel, *Beitr. path. Anat. u. allg. Path.*, 1916, lxi, 564.
42. Epstein, A. A., and Lande, H., Studies on blood lipoids. I. The relation of cholesterol and protein deficiency to basal metabolism, *Arch. Int. Med.*, 1922, xxx, 563.
43. Zuntz, L., Über den Einfluss der Kastration auf den respiratorischen Stoffwechsel, *Deutsch. Z. Chir.*, 1908, xcv, 250; Weitere Untersuchungen über den Einfluss der Ovarien auf den respiratorischen Stoffwechsel, *Arch. Gynäk.*, 1912, xcvi, 188.
44. Marine, D., The present status of the functions of the thyroid gland, *Physiol. Rev.*, 1922, ii, 521.
45. Korentschewsky, W. G., Die Beziehungen zwischen Schild- und Keimdrüsen in Verbindung mit deren Einfluss auf den Stoffwechsel, *Z. exp. Path. u. Therap.*, 1914, xvi, 68.
46. Korenchevsky, V., The sexual glands and metabolism. I. Influence of castration on nitrogen and gaseous metabolism, *Brit. J. Exp. Path.*, 1925, vi, 21.
47. Loewy, A., and Kaminer, S., Ueber das Verhalten und die Beeinflussung des Gaswechsels in einem Falle von traumatischem Eunuchoidismus, *Berl. klin. Woch.*, 1916, liii, 1123.
48. Okintschitz, L., Ueber die gegenseitigen Beziehungen einiger Drüsen mit innerer Sekretion, *Arch. Gynäk.*, 1914, cii, 333.
49. Demel, R., Jatrou, S., and Wallner, A., Beziehungen der Ovarien, Nebennieren und des Thymus zur Thyreoidea bei Ratten, *Mitt. Grenzgeb. Med. u. Chir.*, 1923, xxxvi, 306.
50. Marine, D., The thyroid gland in relation to gynecology and obstetrics, *Surg., Gynec. and Obst.*, 1917, xxv, 272.
51. Marine, D., and Lenhart, C. H., Further observations and experiments on the so-called thyroid carcinoma of the brook trout (*Salvelinus fontinalis*) and its relation to endemic goitre, *J. Exp. Med.*, 1911, xiii, 455.

52. McCarrison, R., The pathogenesis of deficiency disease. No. X. The effects of some food deficiencies and excesses on the thyroid gland, *Indian J. Med. Research*, 1920, vii, 633.
53. Mellanby, E., and Mellanby, M., The experimental production of thyroid hyperplasia in dogs, *J. Physiol.*, 1921, lv, p. vii.
54. Marine, D., Etiology and prevention of simple goiter, *Medicine*, 1924, iii, 453.
55. Shapiro, S., and Baumann, E. J., unpublished data.
56. Rémond, A., and Colombiès, H., and Bernardbeig, J., Cholestérinémie thyro- et parathyroïdienne. Le rôle de la parathyroïde dans le parallélisme de l'azotémie résiduelle et de la cholestérinémie, *Compt. rend. Soc. biol.*, 1924, xci, 445.
57. Heckscher, H., Untersuchungen über den Fett-Cholesterin-Gehalt des Blutes bei thyreoidectomierten Pferden, *Biochem. Z.*, 1925, clviii, 417; Über die Fett-Cholesterin-Menge des Blutes bei Kretinen, *Biochem. Z.*, 1925, clviii, 422.
58. von Eiselsberg, A., Über Wachsthums-Störungen bei Thieren nach frühzeitiger Schilddrüsen-Exstirpation, *Arch. klin. Chir.*, 1894–95, xlix, 207.
59. Pick, E. P., and Pineles, F., Ueber die Beziehungen der Schilddrüse zum Gefässsystem, *Verhandl. Kongr. inn. Med.*, 1908, xxv, 360.
60. Heyn, F., Ein Beitrag zur Lehre vom Myxödem, *Arch. Psychiat.*, 1906, xli, 49.
61. Goldberg, S. A., and Simpson, S., Certain pathological tissue changes in thyroidectomized sheep, *Proc. Soc. Exp. Biol. and Med.*, 1924, xxi, 567.
62. Luden, G., Studies on cholesterol: V. The blood cholesterol in malignant disease and the effect of radium on the blood cholesterol, *J. Lab. and Clin. Med.*, 1918, iv, 849.
63. Leupold, E., Die Bedeutung des Cholesterinstoffwechsels für die weiblichen Keimzellen, *Verhandl. deutsch. path. Ges.*, 1923, xix, 161; Leupold, E., and Seisser, F., *Arch. Gynäk.*, 1923, cxix, 552.
64. Baumann, E. J., unpublished data.
65. Baumann, E. J., and Holly, O. M., The relation of lipoids to suprarenal physiology. I. The cholesterol and lipoid phosphorus contents of the blood of rabbits before and after suprarenalectomy, *J. Biol. Chem.*, 1923, lv, 457.
66. Marine, D., Manley, O. T., and Baumann, E. J., The influence of thyroidectomy, gonadectomy, suprarenalectomy, and splenectomy on the thymus gland of rabbits, *J. Exp. Med.*, 1924, xl, 429; Marine, D., Relation of suprarenal cortex to thyroid and thymus glands, *Arch. Path. and Lab. Med.*, 1926, i, 175.
67. Shapiro, S., and Seecof, D. P., The influence of experimental atherosclerosis upon the systolic blood pressure in rabbits, *J. Lab. and Clin. Med.*, 1925, x, 826.
68. Anderson, H. C., Demonstration of an instrument for taking repeated blood pressures in rabbits, with report of some experiments, *Proc. Soc. Exp. Biol. and Med.*, 1923, xx, 295.
69. Aschoff, L., Lectures on pathology, New York, 1924.