TABLE III. Activities of Lipid Fractions from Incubation Media of Ascites Tumor Cells Which Had Been Incubated with Palmitic-1-C^{14} Acid.

Fraction	0 min.†	10 min.†	20 min.†	30 min.†	60 min.‡
Sterol ester	.1§(.3)‖	— (.2)	.1 (.2)	.1 (.4)	.8 (1.9)
Triglyceride	.1 (.6)	.1 (.7)	— (.5)	.1 (.8)	.4 (2.0)
Fatty acid*	97.7 (85.1)	98.4 (86.3)	97.0 (78.3)	96.5 (78.7)	71.6 (30.5)
Sterol	.3 (3.2)	.1 (1.6)	.5 (3.3)	.2 (2.2)	2.8 (3.5)
Diglyceride	.1 (.8)	.1 (1.2)	.3 (1.5)	.2 (2.2)	8.4¶(19.9)
Monoglyceride	.2 (1.1)	.1 (1.4)	— (.4)	.1 (1.1)	9.6¶(10.4)
Phospholipid	1.5 (8.9)	1.1 (8.6)	2.2 (15.8)	2.9 (14.6)	6.4 (31.9)

* Calculated from weight and activity retained on alumina column.
† Means of 3 determinations.
‡ Only 1 determination.
§ Relative specific activity (specific activity of fraction/sum of specific activities).
‖ Values in parentheses represent % of total activity.
¶ Values are probably not reliable because of extremely small amount of material (less than 1 mg) in each case.

to any extent. Triglyceride does not appear to be either absorbed or released and sterol ester may behave similarly. Thus, it is possible that only the more polar lipids can readily pass through the cell wall.

Further studies with labeled lipids should serve to amplify these indications.

Summary. Ehrlich ascites tumor cells were incubated in ascitic fluid with albumin-palmitate-1-C^{14} complex and were then washed and incubated for varying time intervals with fresh media. Lipids of both cells and media were extracted, separated chromatographically and counted. Results show a decrease of cell free fatty acid with increases in all lipids, especially triglyceride. The major lipid synthesized is phospholipid, which, however declines after 30 minutes. Only phospholipid is released into the medium to any extent.

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Stimulation of Neurosecretion in Shark Embryos by Thyroid Hormones.* (25699)

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The principal physiological role of the hypothalamic neurosecretory system appears to be integration of pituitary gland with certain environmental changes(1). Since this integrative mechanism is involved in such phenomena as seasonal breeding it is not surprising that it is known to differentiate relatively late in the life cycle. In both rats(2) and teleost fishes(3) such differentiation, physiologically and morphologically, appears incomplete until post-embryonic life. Recently, while studying morphological features of the hypothalamic neurosecretory apparatus of shark (*Squalus suckleyi*) embryos, we found that its differentiation is hastened by treatment with thyroid hormones. Since nothing now is

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known of factors which influence development and differentiation of this important system, it seemed to us worthwhile to report this fact apart from the detailed anatomical description.

Methods. About 75 "pup" embryos (length 185 to 210 mm) and about 30 "candle" embryos (length, 61 to 69 mm) of the ovoviviparous shark, *Squalus suckleyi* were removed from uteri of females caught at Friday Harbor, Wash., in Aug., 1958, and kept in glass bowls in groups of 4 or 5 in slowly flowing sea water at 12°-14°C. The embryos of this species spend approximately 2 years *in utero*. The "pups" used were within a month of parturition, and the "candle" embryos about 13 months. Each in a group of 4-5 embryos in one bowl received the same treatment, consisting of intraperitoneal injections of 0.05 cc of 0.8% NaCl solution (control), or this volume of salt solution containing 50 μg propyl thiouracil, 10 μg L-thyroxine, 100 μg L-thyroxine, 100 μg triiodothyronine, 10 μg triiodothyroacetic acid or 100 μg triiodothyroacetic acid. Animals were injected every other day until killed after 2 or 3 weeks of such treatment. Brains of embryos were fixed in Bouin's fluid and serial sections stained with Gomori's chromalum haematoxylin-phloxine (CHP), or aldehyde fuchsin (AF), or by periodic acid-Schiff reaction (PAS).

Results and Discussion. In almost "full-term" embryos a single pair of small neurosecretory centers was found, the preoptic nuclei. Cell bodies of constituent neurones contained very fine granules stainable with all 3 staining procedures. Axones from bilateral centers, also containing stainable neurosecretory granules extended as a preoptico-hypophyseal tract into the neurohypophysis. In the neurohypophysis numerous endings of the secretory axones stained strongly with various stains showing accumulations of stainable material, particularly adjacent to small blood vessels. There was no clear difference in apparent amount of neurosecretion, or in its pattern of distribution between control embryos and those treated with thyroid hormones.

In younger "candle" control embryos no stainable neurosecretory material was discernible in the preoptic nuclear area, or in any of the axones between this area and the neurohypophysis. In the neurohypophysis careful study revealed only sparsely distributed droplets of neurosecretion in the endings of axones. In embryos treated with any of the thyroid hormones there was in almost every instance an increase in number of AF-stained droplets in the neurohypophysis; in most cases numbers of such droplets were more than doubled. An even more striking change in thyroid-treated candle embryos was in the appearance of numerous small droplets in length of the preoptico-hypophyseal neurosecretory tract, running posteriorly in the infundibular floor, above the prolonged tubular adenohypophysis. There was no corresponding change in the preoptic nuclear area. Histological study made of the skin, digestive tract, and respiratory organs of the thyroid hormone treated candle stage embryos revealed no difference from control or propyl thiouracil treated specimens.

It is clear that in the pituitary and hypothalamus of the candle stage shark embryo only the first signs of preoptic nuclear neurosecretory activity can be detected. Between this stage and the "near-term" pup embryo an important degree of differentiation in this system occurs, both quantitatively and qualitatively. Treatment of younger embryos with thyroxine, triiodothyronine, or triiodothyroacetic acid accelerated the differentiation of the hypothalamic neurosecretory apparatus.

Although thyroid hormone has not been known before to affect differentiation of hypothalamic neurosecretion, it is well known as a stimulant of structure and functional differentiation in other parts of brains of amphibian tadpoles and young mammals(4,5,6). The interesting possibility raised by these findings is that thyroxine may also be a stimulant of the adult hypothalamic neurosecretory system. If so, this would explain at least partially, the synergism between thyroid hormone and such adenohypophysis-controlled phenomena as growth and gonadal development.

Summary. In embryos of the shark, *Squalus suckleyi*, differentiation of hypothalamic neurosecretory system is accelerated by treatment with thyroxine, triiodothyronine or

triiodothyroacetic acid. Early accumulation of stainable neurosecretory granules is stimulated in the preoptico-hypophyseal fibre tract, and in the neurohypophysis.

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Further Studies on Passive Transfer of Protection Against Lethality of Endotoxin (25700)

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We have recently described passive transfer, by serum or plasma of endotoxin-tolerant rabbits, of protection against lethality of homologous and heterologous endotoxin in the mouse, and of tolerance to pyrogenicity of endotoxin in the rabbit(1,2). Passive transfer from no-longer tolerant donors, after reticuloendothelial system blockade, and failure to obtain protection when tolerant serum and endotoxin challenge are mixed and given as a single injection, were also demonstrated (2,3). These findings argue against mediation of tolerance by antibody, circulating or fixed. Like endotoxin tolerance itself, transfer appears related to the demonstrated stimulation of RES of recipient(3). Investigations to identify the humoral mediator have eliminated certain obvious possibilities. The present report describes experiments ruling out adrenal cortical hormone, properdin, and phagocytosis-promoting factor. Studies showing protection afforded by normal donor serum under limited experimental conditions, and effect of heat treatment of donor blood are included.

Materials and methods. Preparation and treatment of donor rabbits and recipient mice, and precautions against pyrogen contamination, were as previously described(1,2). Endotoxins, Difco lipopolysaccharides of *S. typhosa* 0901 and *E. coli* 0127:B8, were given *i.p.* for challenge. Aliquots of donor serum were adjusted to pH 5.5 with N HCl, placed into boiling water bath 5 minutes, filtered, and the coagulum washed with saline. The combined filtrate was brought to original serum volume and adjusted to pH 7 with N NaOH. Sera were treated with zymosan (Mann Res. Labs, Lot #4393) both for preparation of "RPb" by 2-stage adsorption at 17 and 37°C, and by exhaustive exposure at 37°C which promotes destruction of C'3, and the zymosan eluted for properdin(4,5). Hydrocortisone was given *i.p.* in 0.2 ml volume at 1 and 5 mg 3 hours before challenge. Adrenal weight was determined for normal and endotoxin-tolerant donor rabbits at sacrifice. Corticosterone was estimated in extracts of normal and tolerant donor plasma by absorption in methanol at 240 mμ. Possible appearance of 17-OH corticoid from stimulation of pituitary-adrenal axis by endotoxin treatment for inducing tolerance was measured by Porter-Silber method(6). Phagocytosis-promoting factor (PPF) of serum was adsorbed on $BaSO_4$ and eluted with citrate(7). In all instances serum or plasma was given *i.p.* in 1 ml volume. Schedules for treatments and challenge are given in the Tables.

Results. The data eliminating adrenal cortical hormone, properdin, and PPF, as mediators of the protective effect of endotoxin-tolerant donor blood administered 3 hours before endotoxin challenge are given in Table I. Steroid, in several thousand times the content of donor plasma (<0.5 μg/ml), did not protect in these experiments. Mean adrenal weight for 19 endotoxin-tolerant rabbits, ob-