served as controls. All caged sparrows were allowed to feed 8 hours daily; the temperature was approximately the same for all these specimens. The experiment extended over a period of 6 weeks; results are shown in Table I.

Sections of the testes from the "light birds" revealed numerous spermatozoa in 9 of the 15 specimens. The gonads of 2 L and 13 L contained only occasional sperms. Slight spermatogenic activity was evident in 12 L and 14 L. Lack of response was noted in 5 L and 9 L. These results confirm those previously reported by the authors.[3]†

The outcome of the experimental studies on the capping of male sparrows is shown in the table. Failure of definite gonadal response is strikingly evident in 6 of the 9 capped birds subjected to additional light. The gonads of the other 3 contained spermatocytes. All control testes were spermatogenically inactive.

Our results indicate that testicular response to light in the sparrow (if experiments are begun in early November) depend largely on reception of the stimulus through the ocular region.‡

9136

Arachidonic and Linolic Acid of the Serum in Normal and Eczematous Human Subjects.*

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Relatively little information is available concerning the content and nature of the various unsaturated fatty acids of the blood. The

† Although Riley recognizes the influence of light in promoting spermatogenic activity experimentally in the quiescent sparrow testis, he concludes that an "intrinsic sexual rhythm" independent of light influence is operative.

‡ According to Whetham, E. O., (*J. Agric. Sci.*, 1933, **23**, 383), "There would seem to be two possibilities concerning the action of light on the reproductive organs by way of the anterior pituitary. Firstly, it may be a quantitative effect on some precursor substance similar to that by which ultra-violet light forms vitamin D; or secondly it may cause stimulation of the anterior pituitary by acting on sensory nerves." Quoting further from Whetham's publication, "Although the rays at the red end of the spectrum have greater penetrating power on animal tissues and so might act on the tissues generally, it would seem more probable in view of the facts at present available that the stimulus acts by way of the nerves associated with color vision."

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work of Hansen[1] and Faber and Roberts[2] which showed that the fat of the serum tends to be less unsaturated in infants with outspoken eczema than in normal healthy infants of similar ages, indicates the necessity of a more detailed study of the constituent fatty acids of the serum. The studies of Burr and Burr[3] and Brown and Burr[4] which point to the essential nature of linolic acid in animal nutrition, naturally bring up the question of the possible significance of this particular fatty acid in relation to the nutrition and metabolism of the human subject. Also, because of its high degree of unsaturation, relatively slight shifts in the content of arachidonic acid of the serum could substantially alter the iodine number of the blood fat. While no specific function of arachidonic acid is known it has been suggested that it plays an important rôle in the intermediary metabolism of fat.[5] The present report deals with the quantitative determination of the insoluble bromides of these particular fatty acids in the sera of normal subjects and patients with eczema.

After aliquots of the lipid extract of the serum were taken for the determination of the cholesterol, the total fatty acids and the iodine absorption capacity of the serum, the remaining portions were pooled in the following classes: a—normal subjects; b—children with eczema; c—eczema cases in clinical remission. Individual samples contained the equivalent of 1 to 3 cc. serum and were obtained from a variable number of subjects, the age groups of which were comparable with the one exception of the group of older patients with active eczema. The extracts of the total lipids were kept in air tight containers in the dark and saved until there was the equivalent of 15 to 50 cc. of serum present. Two sets of samples were determined for each class mentioned. The following technique for the determination of arachidonic and linolic acids was used:

The solvent was first evaporated from the serum extract under vacuum at a temperature below 65°C. and the lipid residue immediately weighed. The necessary amount of alcoholic KOH was added and the lipids were saponified for 30 minutes on a steam bath. After saponification, the soaps were quickly cooled, extracted with ether to remove the unsaponifiable material, and then acidified with dilute HCl to free the fatty acids.

The fatty acids were removed from the acidified solution by 3

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[1] Hansen, Arild E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1198.

[2] Faber, H. K., and Roberts, D. B., *J. Pediat.*, 1935, **6**, 490.

[3] Burr, Geo. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345; 1930, **86**, 587.

[4] Brown, Wm. R., and Burr, Geo. O., Presented at Am. Chem. Soc., Section of Biol. Chem., Kansas City, Missouri, April 16, 1936.

[5] Wesson, L. G., *J. Biol. Chem.*, 1925, **65**, 235.

washings with peroxide-free ether and the ether solution containing the fatty acids then washed free from the HCl with distilled water, using a separatory funnel. The bulked aqueous washings were finally extracted with ether to recover the traces of fatty acids carried into the aqueous phase and this solution again washed free of HCl with water before being added to the fatty acid sample. The amount of fatty acid loss with this technique was found to be negligible.

All traces of ether and water were removed at 65°C. under vacuum, and the dried fatty acids then taken up in petroleum ether, transferred to 15 cc. centrifuge tubes and the insoluble material centrifuged out of solution. The petroleum ether soluble substances were then transferred to a 25 cc. Erlenmeyer flask, the solvent completely removed under vacuum and weighed.

The weighed fatty acids were next dissolved in about 5 cc. anhydrous ether, chilled below 0°C. and brominated with dry bromine gas. After 12 hours' storage at —18°C. the brominated acids were returned to a centrifuge tube, care being taken to remove all traces of the precipitated bromides from the Erlenmeyer flask by the use of additional anhydrous ether. The excess wash ether was removed from the tubes by evaporation with an electric fan.

When the ether solution had been reduced to approximately 5 cc., the precipitate was centrifuged out. This polybromide precipitate was washed until snow white, using one cc. of anhydrous ether for each washing. Usually 3 or 4 washings were required for this purpose. The polybromides were then completely freed of solvent, weighed and calculated as arachidonic acid, the substance indicated by the bromine content of the precipitate, as determined by the Willard-Thompson method.[6]

The ether solution containing the remainder of the brominated fatty acids was then titrated with dilute sodium sulphite to remove the free bromine and the solution evaporated off by an electric fan. When the flask contents were completely water-free (usually about 48 hours), they were extracted with anhydrous ether. The ether solution was centrifuged to remove suspended material, transferred to a second centrifuge tube, and the ether then evaporated off. Five cc. petroleum ether was added to the contents of the centrifuge tube and the mixture stirred, stoppered, and stored for at least 12 hours in a refrigerator. Upon removal from the refrigerator, the precipitate of tetrabrome-linolic acid was centrifuged from solution and washed with petroleum ether, the same technique as described for the polybromides being used.

[6] Willard, H. H., and Thompson, J. J., *J. Am. Chem. Soc.*, 1930, **52**, 1893.

In these precipitations it was found that if the amount of solvents used was reduced to the minimum requirement to carry the precipitate, the loss due to the precipitate solubility was unweighable with a high-grade analytical balance.

Results. The results are summarized in Table I and are expressed in percentage of the total fatty acid present.

TABLE I.
Content of Linolic and Arachidonic Acids in Pooled Samples of the Sera from Normal and Eczematous Subjects.

Type	Arachidonic Acid (Polybromides) % of Total Fatty Acid	Linolic Acid (Tetrabromides) % of Total Fatty Acid
Normal		
35 subjects	2.83	4.80
12 "	2.90	5.20
Eczema (active)		
18 subjects (younger group)	1.34	3.20
8 " (older group)	1.60	4.20
Eczema (treated—in remission)		
12 subjects	1.40	4.80
5 "	0.76	5.40

As regards the two samples of blood from the normal subjects, there is a striking agreement in the content of the arachidonic and linolic acid, and the quantity of these acids present in these samples is very similar to that found in a large sample of blood from a normal adult human being.[7] The arachidonic acid value is comparable to that reported by Tängl[8] for ox blood serum. In both samples of serum from the eczematous subjects the content of both linolic and arachidonic acids was definitely diminished. This finding confirms the previously reported observations[1, 2] that the total fatty acids of the serum from children with eczema have a lower iodine number than do those of normal children of comparable ages, also, that the difference in iodine number is not as marked in older children with eczema as in the younger age group.[9] The possible significance of these findings in relation to factors such as age, diet and infection, which are known to affect the degree of unsaturation of the serum lipids, is discussed in a current publication.[9]

The specimens from the patients with eczema in remission were obtained from those who were in good condition clinically, most of them having been treated by the administration of raw linseed oil or corn oil internally for variable lengths. However, part of the

[7] Brown, Wm. R., Hansen, Arild E., Burr, G. O., and McQuarrie, Irvine, PROC. SOC. EXP. BIOL. AND MED., in press.

[8] Tängl, H., *Biochem. Z.*, 1930, **226**, 180.

[9] Hansen, Arild E., *Am. J. Dis. Child.*, 1937, in press.

sample was obtained from patients who had been treated with local application of ointments, containing crude coal tar. The values of linolic acid were definitely higher in these treated groups, being in the same range as found for normal subjects. It seems only reasonable to believe that this increase in the linolic acid content is explained by the fact that diets of these subjects for the most part contained fats which were rich in linolic acid. This finding, however, does not prove that the return to normal values of the linolic acid content is concerned with changes in the clinical picture noted in these cases. There was no effect on the arachidonic acid content of these specimens, although in one instance the values were definitely lower. Our conjecture is that linolic acid (or at least the unsaturated fatty acids), which may be necessary for normal skin nutrition, is probably one of the many factors disturbed in eczema.

Summary and Conclusions. Linolic acid (tetrabromides calculated as such) is present in human serum to the extent of about 5% of the total fatty acids, and arachidonic acid (polybromides calculated as such) about 3% of the total fatty acids in pooled samples of blood serum from normal children. The content of both these fatty acids is definitely diminished in children with eczema. In those cases of active eczema which were clinically cured either by the internal administration of oils rich in the unsaturated fatty acids or by the local application of ointments containing crude coal tar, or both, the arachidonic acid was not increased while the linolic content was the same as that found in normal subjects. These findings indicate a possible reason for the low iodine number of the serum fatty acids in eczema and further suggest that a disturbance in the metabolism of the unsaturated fatty acids may be one of the many factors at fault in this condition.

9137

Determination of Staphylococcal Types by Fermentation of Mannite.*

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The recent demonstration that different strains of staphylococci elaborate chemically distinct carbohydrates[1] has made it possible to

* Conducted under a grant from the Commonwealth Fund of New York.

[1] Julianelle, L. A., and Wieghard, C. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 947; also, *J. Exp. Med.*, 1935, **62**, 23.