significantly, on the knowledge that incubation periods of such duration of suspected foods are frequently found in food poisoning outbreaks. It was found that 10 to 25 fold increases of initially large inoculums had taken place in the vehicle foods prior to ingestion.

It is of interest that the number of organisms which were found to produce experimental symptoms in this study is of the same order as that which Kelly and Dack[5] found able to produce symptoms of gastroenteritis when a strain of *Staphylococcus aureus* was fed. These authors produced illness in one volunteer with 48×10^9 organisms and in another with 69.3×10^9. In the present study the symptom producing dose of *Streptococcus faecalis* ranged from 22.7×10^9 to 148×10^9 organisms. This comparison is noteworthy since staphylococcal food poisoning is believed to be caused by an "enterotoxin" while Sherman, Gunsalus, and Bellamy[6] suggest the possibility that the toxic principle of *Streptococcus faecalis* poisoning may be tyramine formed by the decarboxylation of tyrosine by this organism. Also perhaps worthy of comment is the fact that like staphylococcal food poisoning, streptococcal food poisoning usually has a short incubation period.

Summary. Symptoms of acute gastric or intestinal disturbance or of both were produced in 6, or possibly 7, of 26 human volunteers who ate foods in which strains of *Streptococcus faecalis* had grown for 5 hours.

Attempts to produce similar symptoms in man with 20-hour cultures were unsuccessful. Kittens were found to be unsatisfactory test animals in feeding experiments with inoculated condensed milk.

Conclusion. The experimental production of acute mild intestinal or gastric disturbance or both in man, tends to confirm the etiological role of *Streptococcus faecalis* in naturally occurring outbreaks of food poisoning.

We are indebted to Drs. William Birnkrant and Morris Greenberg of the Bureau of Preventable Diseases who conducted the physical examinations and diagnosed the illnesses which occurred.

[5] Kelly, F. C., and Dack, G. M., *A. J. P. H.*, 1936, **26**, 1077.

[6] Sherman, J. M., Gunsalus, I. C., and Bellamy, W. D., 57th Annual Report, N. Y. State Col. Agr., Cornell University Agr. Exp. Sta., 1944.

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Distribution and Nature of the "Antigen" Responsible for Experimental Meningoencephalomyelitis in the Guinea Pig.*

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Previous work has shown that a meningoencephalomyelitis, sometimes with foci of demyelinization, may occur in monkeys, guinea pigs, and rabbits 2 to 9 weeks following the subcutaneous or intramuscular injection of brain tissue mixed with acid-fast bacilli in a water-in-oil emulsion.[1-6] In con-

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[1] Morgan, I. M., *J. Exp. Med.*, 1947, **85**, 131 (preliminary report in *J. Bact.*, 1946, **51**, 53).

[2] Kabat, E. A., Wolf, A., and Bezer, A. E., *J. Exp. Med.*, 1947, **85**, 117 (preliminary report in *Science*, 1946, **104**, 362).

[3] Wolf, A., Kabat, E. A., and Bezer, A. E., *J. Neuropath. and Exp. Neur.*, 1947, **6**, 333.

[4] Freund, J., Stern, E. R., and Pisani, T. M., *J. Immunol.*, 1947, **57**, 179.

[5] Kopeloff, L. M., and Kopeloff, N., *J. Immunol.*, 1947, **57**, 229.

firmatory work in this laboratory an ascending paralysis has developed in slightly more than half of some 90 guinea pigs following the single subcutaneous injection of one cc of similar emulsions containing guinea pig, rabbit, human, or beef brain suspended 1:20 in 0.9% saline. The illness usually began 15 to 31 days after injection, although it was delayed as long as 74 days in one case; the animals usually died within 10 days, although some recovered and others remained paralyzed for one to 2 months. Histological examinations of brain and spinal cord removed from about 30 paralyzed guinea pigs in every case revealed a patchy or diffuse perivascular inflammation, predominantly lymphocytic, in the leptomeninges and entire central nervous system, most concentrated in the white matter. The evidence that this disease may represent an allergic reaction has been recently reviewed.[7] Further experiments now to be described have shown that the "antigen" responsible for this experimental disease is present in certain phosphatide-like extracts of brain tissue, in a preparation of "purified brain lipids,"[‡] and in optic nerve, which contains only white matter.

To learn about the nature of the "antigen," various extracts of whole moist beef or human brain were made according to the outline of Page,[8] using acetone, cold petrol ether, cold 95% alcohol, ether, and warm pyridine. In all, 5 different preparations of phosphatide-fractions were made, these being insoluble in acetone and soluble in cold petrol ether and ether; 4 of these were further fractionated into a lecithin-component (soluble in cold 95% alcohol) and a cephalin-portion (insoluble in cold 95% alcohol). Eight different preparations of other fractions of brain were made. These included cholesterol and other substances repeatedly soluble in acetone; cerebrosides and sphingomyelin or a mixture of these referred to as "protagon,"[9] which were insoluble in acetone, cold petrol ether, and cold 95% alcohol but soluble in pyridine warmed to 30°-45°C and in alcohol warmed to 80°C; and residual material which was insoluble in acetone, cold petrol ether, and warm pyridine. Each of these fractions was made up to an approximately 5% suspension in 0.9% saline; an emulsion was then made by mixing two parts of this suspension in a Waring blendor with two parts of Bayol-F[§] containing 2.5 mg/cc heat-killed tubercle bacilli and one part of Falba.[¶] Each emulsion was then tested by injecting one cc subcutaneously into each of 3 guinea pigs. Four of the 5 emulsions containing the phosphatide-fractions produced paralysis in 6 of 13 guinea pigs injected, whereas none of the 8 emulsions containing the other fractions produced any illness in 22 guinea pigs injected.

As a check on the lipid nature of the "antigen," an emulsion of "purified brain lipids"[‡] was made and injected into 5 guinea pigs. Nine days following a second injection 3 months later, one guinea pig developed a characteristic paraplegia. None of 5 guinea pigs similarly injected twice with an emulsion containing "purified phosphatidyl-serine"[9] became ill.

To learn about the distribution of the "antigen," optic nerves, which are composed only of white matter, were procured post-mortem from 2 human beings, care being taken to avoid the perichiasmal tissue. One of 3 guinea pigs injected with an emulsion made from this tissue became paralyzed.

In summary, it would seem that the "anti-

[6] Morrison, L. R., *Arch. Neur. and Psych.*, 1947, **58**, 391.

[7] Stevenson, L. D., and Alvord, E. C., Jr., *Am. J. Med.*, 1947, **3**, 614.

[‡] A preparation generously supplied by Dr. Jordi Folch-pi, McLean Hospital, Waverley, Mass., and said to contain practically all of the lipids present in whole brain and to be free of protein and carbohydrate.

[8] Page, I. H., *Chemistry of the Brain*, Charles C. Thomas, Springfield, 1937, p. 54.

[9] MacLean, H., and MacLean, I. S., *Lecithin and Allied Substances, the Lipins*, Longmans, Green & Co., Ltd., London, 1927, p. 125.

[§] A light paraffin oil, obtained through the courtesy of Mr. K. L. Patterson, Stanco, Inc., New York City.

[¶] An adsorption base said to be a mixture of oxycholesterine and cholesterines derived from lanolin (Pfaltz and Bauer, Inc., New York City).

[9] Also supplied by Dr. Folch-pi and described in *J. Biol. Chem.*, 1942, **146**, 35.

gen" responsible for the meningoencephalomyelitis is a phosphatide-like material present in the white matter of the central nervous system. Its properties serve to differentiate it from 4 of the 5 haptens thought to be present in brain.[7] "Neurokeratin"[10] has been eliminated by previous workers;[5] "protagon" and "sphingomyelin"[11] are included in the non-phosphatide fractions tested in the present experiments and found to be inactive; and the hapten thought to be present in gray matter[11,12] is obviously not necessary since it has now been found that white matter (optic nerve) contains the "antigen." Whether the "antigen" is the same as the "alcohol-soluble brain hapten"[11,12,13] must await further studies.

[10] Bailey, G. H., and Gardner, R. E., *Am. J. Hyg.*, 1942, **36**, 205.

[11] Schwab, E., *Z. f. Immunitätsforsch. u. exp. Therap.*, 1936, **87**, 426.

[12] Reichner H., and Witebsky, E., *Ibid.*, 1934, **81**, 410.

[13] Rudy, H., *Biochem. Z.*, 1933, **267**, 77.

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Thiouracil and Mammary Growth.*†

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There are a number of reports in the literature which would appear to indicate that the mouse and the rat may respond differently, as regards mammary development and responsiveness, during experimentally induced hypothyroidism.

Mixner and Turner[1] have reported an increased mammary responsiveness of ovariectomized female mice to estrogen and progesterone treatment when thyroxin was simultaneously administered, while a decreased responsiveness followed thyroidectomy. Mixner[2] later found that thiouracil administration had an effect similar to thyroidectomy in decreasing mammary responsiveness. Morris *et al.*, found considerable mammary atrophy in virgin female mice fed thiourea[3] and thiouracil[4]. Gardner[5] observed mammary growth in male mice fed desiccated thyroid, but the effect was dependent upon intact testes.

From these reports it would appear that, in the mouse, experimentally induced mild hyperthyroidism is conducive to enhanced mammary responsiveness, while the opposite is true of hypothyroidism.

In the rat, however, Leonard and Reece,[6] and Smithcors and Leonard[7] have found that thyroidectomy of both male and female rats caused an enhanced development of the mammary gland. Thyroidectomy also increased the effectiveness of administered estrogen or testosterone propionate in stimulating mammary alveolar development. Chamorro[8,9] has also reported that thyroid-

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† The diethylstilbestrol and its dimethyl ether used in this investigation were generously provided by Merck and Co., Rahway, N.J., and the thiouracil by Lederle Lab., Pearl River, N.Y.

[1] Mixner, J. P., and Turner, C. W., *Endocrinology*, 1942, **31**, 345.

[2] Mixner, J. P., *J. Dairy Science*, 1947, **30**, 578.

[3] Morris, H. P., Dubnik, C. S., and Dalton, A. J., *J. Nat. Cancer Inst.*, 1946, **7**, 159.

[4] Morris, H. P., Dubnik, C. S., and Dalton, A. J., Exhibit Abstract, Fourth Internat. Cancer Res. Congress, Sept. 2-7, 1947, St. Louis.

[5] Gardner, W. U., *Endocrinology*, 1942, **31**, 124.

[6] Leonard, S. L., and Reece, R. P., PROC. SOC. EXP. BIOL. AND MED., 1941, **28**, 65.

[7] Smitheors, J. F., and Leonard, S. L., *Endocrinology*, 1942, **31**, 454.

[8] Chamorro, A., *C. R. Soc. Biol.*, 1946, **140**, 499.