and one day preceding inoculation with virus; 2 rabbits received 2 injections of 5 cc. each, 2 days and again 1 day preceding the inoculation. Eight rabbits received a single injection of 2 cc. per kilo of serum 1, 2, 3, 4, 7, 14, 21, and 35 days, respectively, preceding inoculation. All but one of the animals receiving serum were protected. This animal had received the serum 35 days previously. All of the controls (4 in number) developed generalized vaccinia in 6 to 7 days.

Another series of animals were given serum injections at varying intervals of time *following* intranasal instillations of virus. Five rabbits received 4 cc. of serum per kilo of animal weight 1, 6, 12, 24 and 36 hours, respectively, after inoculation. All were protected, while the controls developed generalized vaccinia on the 6th day. Four additional rabbits received the same dose of serum 24, 48, 72 and 96 hours, respectively, following inoculation. The 2 animals which received the serum within 2 days survived, while the 2 which received the serum later developed generalized vaccinia on the 6th day.

The results suggest that passive immunization is considerably more effective in protecting rabbits against generalized vaccinia following intranasal instillation of virus than in protecting monkeys against intranasal inoculation with poliomyelitis virus. This harmonizes with evidence already available[3] that in poliomyelitis, the virus is transmitted from the olfactory mucosa to the central nervous system by the olfactory nerves essentially out of the reach of immune substances in the blood plasma.

8595 C

Dietary Production of Specific Syndrome of Deficiency in Vitamin B_6.*†

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As a result of recently reported work from many laboratories it appears certain that the factor formerly known as vitamin G

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(B_2) is a complex—one member of which is flavin and the other a relatively heat- and alkali-stable factor. Lack of this second factor leads to a "specific" form of dermatitis described in detail by Chick and co-workers,[1] György[2] and others.

Some difficulty, however, has been noted in various laboratories in inducing this particular dermatitis. The Sherman-Bourquin diet[3] has been found very successful in general, particularly when the added precaution has been taken of using the Page anticoprophagy harness.[4] However, György noted that different results were obtained when different lots of wheat were used. Hogan and Richardson[5] have shown that cornstarch contains an alcohol-soluble substance which cured dermatitis in their rats when they were given a diet of casein, sucrose, salts, cod liver oil and cellulose, plus an irradiated aqueous extract of yeast. If the diet contained starch in place of sucrose no dermatitis developed. The condition was also cured by wheat germ oil.

One of us has used the Sherman-Bourquin diet over a period of some years and has not had difficulty in inducing the dermatitis and cessation of growth. However, several attempts to repeat this in this laboratory have proved unsuccessful, even when harnesses were used. The rats when receiving no additional supplement to the basal diet ("negative control rats") continued to grow and showed no skin abnormalities. It was then suspected that the wheat was unusually rich in the B_2 factors and that they were extracted along with vitamin B_1 by the alcohol. We were fortunate in receiving from Merck and Company some vitamin B_1 crystals as well as a highly potent concentrate from rice polish. When the Sherman-Spohn diet[6] (deficient in all known B factors) was substituted, plus the B_1 concentrate, the incidence of dermatitis was greater. Practically all animals developed skin lesions after receiving the diet for 6 to 8 weeks.

Booher and co-workers[7] have shown that the flavin component of vitamin B_2 is extracted from whey by hot 95% alcohol. When casein is extracted with hot concentrated alcohol (as in the Sher-

[1] Chick, H., Copping, A. M., and Edgar, C. E., *Biochem. J.*, 1935, **29**, 722.

[2] György, P., *Biochem. J.*, 1935, **29**, 741.

[3] Bourquin, A., and Sherman, H. C., *J. Am. Chem. Soc.*, 1931, **53**, 3501.

[4] Page, J. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 87.

[5] Hogan, A. G., and Richardson, L. R., *J. Nut.*, 1935, **8**, 385; *Nature*, 1935, **136**, 186; *Science*, 1936, **83**, 17.

[6] Sherman, H. C., and Spohn, A., *J. Am. Chem. Soc.*, 1923, **48**, 2791.

[7] Booher, L. E., *J. Biol. Chem.*, 1932, **102**, 39; Booher, L. E., Blodgett, H. M., and Page, J. W., *J. Biol. Chem.*, 1934, **107**, 599.

man-Munsell[8] method for its preparation for use in vitamin A-free diets) the resultant alcohol is always fluorescent. The usual method for extraction of casein for the Sherman-Spohn diet is by the use of cold 60% alcohol. We felt that, due to the extractability of the fluorescent material with hot concentrated alcohol, such treatment might be advisable. We therefore adopted the method of Sherman and Munsell and followed this by 2 cold 60% alcohol extractions, with quite satisfactory results.

Young rats at 21 days of age weighing about 45 gm., when given this modified Sherman-Spohn diet plus a vitamin B_1 concentrate (in amounts which induce a gain of 8 to 10 gm. weekly in rats receiving a vitamin B_1 deficient diet) gained 10 to 15 gm. in about 2 weeks. By the end of this period about 35% of the rats showed rough paws with inflammation between the toes. These symptoms became progressively worse. In order to compare the results obtained with this diet with results from other laboratories, it was necessary to feed the deficient rats supplements similar to, if not identical with, the materials used by other workers. To this end we prepared alcoholic and aqueous extracts of brewer's yeast and aqueous extracts of fresh hog liver. These were treated with Fuller's earth according to the methods used in the preparation of flavin by other investigators. The adsorbates and resulting filtrates were fed separately or in combination. Results from these experiments will be reported in detail at a later date. However, of 35 rats receiving no supplement (except vitamin B_1), or receiving the material adsorbed by Fuller's earth from the yeast or liver extracts, 30 showed raw and edematous paws, swollen ears and sores around the nose and mouth by the end of 5 weeks. There was no alopecia. Death has occurred in some cases as early as the fourth week. Care was taken to prevent access to feces, but the harnesses were not used. In confirmation of Chick and coworkers it seemed that feeding of the adsorbed material (the flavin component) intensified the symptoms. The filtrates from the yeast extract, even after autoclaving for 1 hour at pH 9, prevented the lesions. The liver filtrate was effective in both curative and prophylactic experiments.

In all of this work cornstarch was used. In order to determine whether the use of sucrose would be more satisfactory, a diet was made up in which sucrose was substituted for the starch. The diet contained 8% butter fat and 2% cod liver oil, and these do not blend with sugar as with starch. The animals became very

8 Sherman, H. C., and Munsell, H. E., *J. Am. Chem. Soc.*, 1925, **47**, 1639.

greasy in appearance and there was some slight alopecia, probably due to irritation. The "specific" skin symptoms, however, did not appear earlier and were no more severe. Death occurred somewhat earlier in the case of the negative control rat, and a greater length of time was required to induce a cure.

Itter and co-workers[9] have correlated alopecia with the absence of the sulfhydryl group and found that feeding of *cysteine*-HCl led to cures. Prunty and Roscoe[10] showed that purified casein might be deficient in cystine, with the result that the growth rate was lessened. They could not cure the "specific" dermatitis with cystine, however. Whether or not our treatment of casein would lower the cystine content to such an extent as to be a factor in our results is being investigated.

It may be concluded that the "specific" dermatitis and cessation of growth can be readily induced in rats receiving a diet containing cornstarch, provided a highly purified and potent vitamin B_1 concentrate is used and the casein is so extracted as to remove the B_2 factors. Whether this dermatitis is identical with that induced by Hogan and Richardson remains to be determined.

8596 C

Veterinary Staphylo-Fibrinolysin.*

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In an examination of 132 local strains of staphylococci† it was found that 80% of all strains originally isolated from internal human lesions are capable of liquefying human fibrin.[1] Approximately 90% of all strains isolated from superficial human infec-

[9] Itter, S., Orent, E. R., and McCollum, E. V., *J. Biol. Chem.*, 1935, **108**, 585.

[10] Prunty, F. T. G., and Roscoe, M. H., *Biochem. J.*, 1935, **29**, 2491.

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† The strains and clinical histories used in these surveys were kindly furnished by the various hospitals, clinics, diagnostic laboratories, and veterinary institutions of the San Francisco Bay Region.

[1] Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 209.