been established less than 18 hours. In accordance with our present concepts this may be taken to indicate that at this time, and possibly even earlier, the melanophore hormone is already being produced and secreted by the developing gland. The morphology of the gland at this period is therefore of some interest. In sections it appears as a mass not differentiated into parts and still showing remnants of a column of cells connecting to the ectoderm. The cells still contain considerable yolk granules, the cytoplasm is scant and does not show granular differentiation (Fig. 3). We are thus led to conclude that melanophore hormone secretion is initiated in the still undifferentiated primordium of the pituitary in Xenopus.

Summary. Observations on the development of the melanophores in normal and hypophysectomized Xenopus show contraction of the melanophores in the latter at a late tail bud stage, about 48 hours after fertilization. The pituitary at this stage is undifferentiated. It is inferred that the secretion of melanophore hormone begins at or possibly before this time.

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Selective Inhibition of Sulfonamide Drugs by Various Media.

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It is now recognized that body fluids, as well as the usual laboratory media for cultivation of bacteria, contain substances which inhibit the action of sulfonamide drugs.[1, 2] It has been shown that different media vary in the amount of inhibiting substances they contain.[2] The question arises whether different sulfonamide drugs are inhibited to the same degree in a given medium. Were this true it might be possible to draw valid conclusions from *in vitro* studies of the comparative bactericidal or bacteriostatic effects of different drugs. In this paper we present some evidence that this assumption may not be justified.

Methods and Materials. Growth curves with stock strains of

[1] Lockwood, J. S., and Lynch, H. M., *J. Am. Med. Assn.*, 1940, **114**, 935.

[2] MacLeod, C. M., *J. Exp. Med.*, 1940, **72**, 217.

Type III and Type V pneumococci were made as in previous studies.[3] In making explants for colony counts, the bacteriostatic effect of sulfonamide drugs was inhibited by the addition of *p*-aminobenzoic acid.[4] The "blood broth" consisted of a meat infusion broth with 1% bacto-peptone (Difco) and 0.05% glucose, to which 1% defibrinated rabbit blood was added. "Liver infusion" was prepared as described by MacLeod,[2] but modified by the addition of 1% of an acid hydrolysate of casein* and suitable buffering salts.

Results. The comparative effects of equal concentrations of sulfathiazole and sulfadiazine on the growth of Type III pneumococci in various media are shown graphically in Fig. 1.

In blood broth, with inocula of about 100 organisms per milliliter, 10 mg % of sulfathiazole are bactericidal in 24 hours. Under identical conditions, sulfadiazine is only slightly bacteriostatic. In horse serum the results are about the same as in blood broth. In human plasma (cell-free citrated blood) sulfadiazine exhibits relatively more bacteriostasis, and sulfathiazole relatively less, than in blood

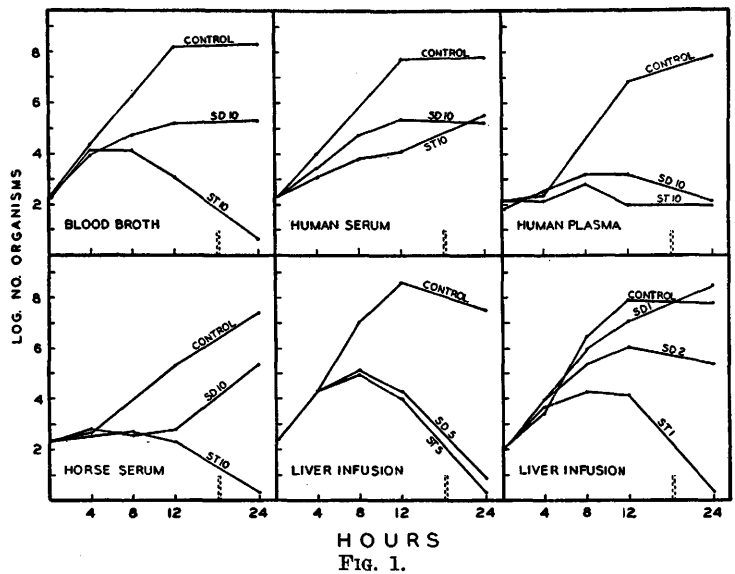

FIG. 1.

Growth curves of Type III pneumococcus in various media. S.D. = sulfadiazine, S.T. = sulfathiazole, and the numbers indicate the concentration of these drugs in mg per 100 ml. Control contains no drug.

[3] Spring, W. C., Jr., Lowell, F. C., and Finland, M., *J. Clin. Invest.*, 1940, **19**, 163.

[4] Strauss, E., Lowell, F. C., and Finland, M., *J. Clin. Invest.*, 1940, **20**, 189.

* Prepared and supplied by Dr. J. Howard Mueller.

broth. On the other hand, in human serum (from clotted blood) sulfathiazole is inhibited to a much greater degree than in any of the other media, while sulfadiazine is about as effective as it is in blood broth or in horse serum. In the liver infusion medium the two drugs are equally bactericidal in concentrations of 5 mg % or more. In this medium, however, sulfathiazole is bactericidal in concentrations as low as 1 mg %, whereas a similar concentration of sulfadiazine is almost without effect.

In Fig. 2 a comparison is made of the effect of sulfathiazole and sulfadiazine on the growth of Type V pneumococci in whole defibrinated blood and in the serum of the same defibrinated blood. In previous tests this subject's blood was found to possess no natural bactericidal action against Type V pneumococci. Here again sulfathiazole was more effective in defibrinated blood than in serum. Sulfadiazine, on the other hand, was only slightly bacteriostatic in both media.

Comment. Various types of *in vitro* tests have been used for the determination of the comparative bactericidal and bacteriostatic efficacy of sulfonamide drugs.[5-8] In studies on the action of sulfapyridine and sulfathiazole on the pneumococcus, both the clinical results and therapeutic experiments in animals correlated fairly closely with *in vitro* results. With sulfadiazine it became evident

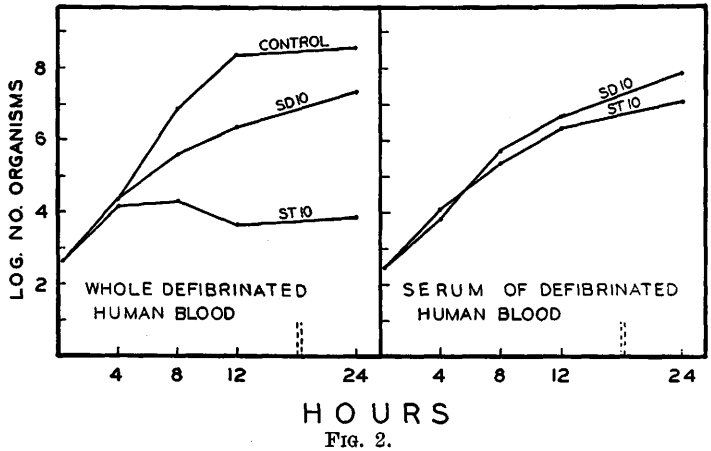

FIG. 2.

Growth curves of Type V pneumococcus in defibrinated blood and in its serum. S.D. = sulfadiazine, S.T. = sulfathiazole, and the numbers represent concentration of the drugs in mg per 100 ml. Control contained no drug.

5 Chu, H. I., and Hastings, A. B., *J. Pharmacol. and Exp. Therap.*, 1938, **63**, 407.

6 Kempner, W., Wise, B., and Schlayer, C., *Am. J. Med. Sci.*, 1940, **200**, 484.

7 Long, P. H., and Bliss, E. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 324.

8 Lowell, F. C., Strauss, E., and Finland, M., *Ann. Int. Med.*, 1940, **14**, 1001.

that the excellent therapeutic results in animals[9] and in patients[10, 11] were at variance with the results obtained *in vitro* with the media usually employed for these tests. The results reported in this paper emphasize the importance of the proper selection of media for the performance of *in vitro* tests. Both sulfathiazole and sulfadiazine are inhibited to various degrees in the media used; moreover, the degree of inhibition of the same drug is not constant in different media. On the basis of activity in blood broth it might be concluded that sulfadiazine is no more active against Type III pneumococci than an equivalent concentration of sulfanilamide. Essentially the same results were obtained by Osgood, using marrow cultures.[12] In a liver infusion medium, however, sulfadiazine is as active as sulfathiazole in concentrations of 5 mg % or more. In lower concentrations in this medium our unpublished observations indicate that it is about as active as sulfapyridine.

Summary. Sulfathiazole and sulfadiazine are inhibited to varying degrees in different media. In blood broth sulfadiazine is selectively inhibited to a greater degree than sulfathiazole. In human serum sulfathiazole is inhibited to a greater degree than it is in blood broth, in human plasma, in defibrinated blood or in horse serum, while sulfadiazine is inhibited to about the same extent in these media. In liver infusion medium sulfadiazine and sulfathiazole are equally effective in a concentration of 5 mg % but sulfathiazole is more effective in lower concentrations. The possible error of attempting to compare therapeutic efficacy of different sulfonamides on the basis of the results of *in vitro* tests is discussed and the importance of the proper selection of media for the performance of *in vitro* tests of sulfonamide drugs is emphasized.

[9] Feinstone, W. H., Williams, R. D., Wolff, R. T., Huntington, E., and Crossley, M. L., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 427.

[10] Flippin, H. F., Rose, S. B., Schwartz, L., and Domm, A. H., *Am. J. Med. Sci.*, 1941, **201**, 585.

[11] Finland, M., Strauss, E., and Peterson, O. L., *J. Am. Med. Assn.*, 1941, **116**, 2641.

[12] Bullowa, J. G. M., personal communication.