vived one killing dose of histamine. These rats increased markedly in weight, and at autopsy the suprarenals showed striking hypertrophy as did the gonads, spleen and thyroid. Nevertheless this was not associated with an increase in the natural resistance to histamine. The natural resistance of normal rats was not appreciably raised by repeated injections of a saline emulsion of anterior hypophysis.

TABLE II.
Effect of Repeated Injections of Anterior Hypophyseal Saline Emulsion on the Natural Resistance of Normal Rats to Histamine Poisoning (Ergamine Acid Phosphate).

No. Rats	Amt. Histamine per kg.	Survived	Died
	Normal rats treated with anterior hypophyseal emulsion.*		
6	1100	2	4
6	1200	1	5
3	1400	0	3
1	1600	0	1
	Normal rats untreated.		
2	900	2	0
4	1000	2	2
4	1100	1	3

* The emulsion was prepared as in the previous experiment. Subcutaneous injections were given in amounts of 1 cc. daily for a period of 14 days prior to the administration of histamine.

Summary. The natural resistance of hypophysectomized rats treated with a crude emulsion of fresh anterior hypophysis during a period of 2 weeks following the operation to subsequent injections of histamine is increased almost to the normal level. The natural resistance of normal rats treated with a crude emulsion of fresh anterior hypophysis during a period of 2 weeks to subsequent injections of histamine is not appreciably increased.

8277 P

Effect of Vitamin A on Proliferation of Fibroblasts.

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The purpose of the experiments reported here was to ascertain whether or not vitamin A is one of the substances needed by fibroblasts for their life and proliferation *in vitro*. Two previous investigators have already attempted to answer this question. Unfortunately, however, they arrived at opposite conclusions. Thus, Bur-

rows[1] compared the change in concentration of vitamin A in chick embryos, as they increased in age, with the change occurring simultaneously in the growth-promoting power of the juice of the embryos. He concluded that vitamin A inhibited growth. Bisceglie,[2] on the other hand, added a trace of the "A factor" to hanging drop cultures of liver and spleen and found it to be growth stimulating. No information was given as to the source of the "A factor", or the extent to which it had been purified.

In the present investigation, crystalline carotene was first tried as a source of vitamin for the cells. No increase in growth was obtained.* Vitamin A was, therefore, prepared from halibut liver oil. Two different concentrates were made. In preparing the first, the oil was saponified, and the vitamin extracted with petroleum ether. The ether was evaporated, and the residue taken up in methyl alcohol. Sterols were then removed by cooling the methyl alcohol solution to 0°C. After this, the vitamin was redissolved in hexane, and more sterols were removed by cooling to —10°C. An oily residue was obtained on evaporation of the hexane. The second preparation was purified to a further extent. In this case, a mixture of CO_2 ice and alcohol was used to freeze out the sterols from the methyl alcohol solution. The residue from the methyl alcohol was then dissolved in pentane and the freezing with CO_2 ice repeated. After this, the vitamin was dissolved in petroleum ether, adsorbed on alumina, and eluted with chloroform.[3] A product of a high degree of purity was thus obtained. For the purpose in hand, it did not seem advisable to attempt any further purification, at least, not until such a time as the vitamin will have been obtained in crystalline form.

In order to bring the vitamin into a form in which it could be utilized by the cells, an attempt was made to dissolve it in serum. Samples of chicken sera were allowed to stand overnight at room temperature with small quantities of the vitamin preparations. When tested by the antimony trichloride reaction,[4] these sera were found to have increased their original vitamin A content approximately 200

[1] Burrows, M. T., *Am. J. Physiol.*, 1926, **77**, 24.

[2] Bisceglie, V., *Arch. Entwiklngsmechn. Organ.*, 1926, **103**, 708.

* Further work is being done to ascertain the reason for the different results obtained with carotene and vitamin A.

[3] These methods of preparation were taken largely from the work of Holmes and his co-workers. Holmes, H. N., Cassidy, H., Hartzler, E., and Manly, R., *Science*, 1934, **79**, 255; Holmes, H. N., Lava, V. G., Delfs, E., and Cassidy, H., *J. Biol. Chem.*, 1932, **99**, 417.

[4] Morris, E. R., and Church, A. E., *J. Biol. Chem.*, 1929, **85**, 477.

times. A second portion of each sample of serum (for use in the control cultures) was likewise kept overnight at room temperature without being exposed to the vitamin.

The "vitamin sera" were then tested for their effect on the growth of heart fibroblasts. Two different strains of fibroblasts were used: one, the 23-year-old strain of Carrel; the other, a new strain that had been derived quite recently from the heart of an embryonic chick. Each culture was divided into 2 equal parts. These were embedded in separate flasks in coagula made of horse plasma.† One of these cultures received the vitamin serum; the other, an equal amount of the control serum. All of them were given a nutrient fluid that contained Witte's peptone, cysteine, hemin, insulin, thyroxine, glucose,[5] the salts of Tyrode solution, and some normal serum.‡ The vitamin and control sera were used in amounts ranging from 0.002 to 0.05 cc. per cc. of medium. The normal serum was given in such amounts as to make the total concentration of serum in the medium equal to 10%.

When used at the highest concentrations, the "vitamin sera" were toxic. At lower concentrations, however, they had a most beneficial effect on the cells. The cells that received the vitamin proliferated much more rapidly than did the control cells. In many instances, they formed colonies 3 times as large as those in the controls. The largest growth was generally obtained when the "vitamin sera" were used as 1% of the medium. In addition to promoting growth, the incorporation of vitamin in the medium prevented the accumulation of large amounts of fat in the cells, a reaction that was quite characteristic of the control cultures. It also enabled the cells to live and proliferate in the artificial medium for a much longer time than they could in the absence of the vitamin. As far as could be observed, the 2 preparations of the vitamin gave identical results, in spite of the differences in the extent to which they had been purified.

† The horse plasma used in these experiments was supplied in an aseptic condition by the New York City Department of Health. The author wishes to express her appreciation of this favor.

5 The constituents used by Vogelaar and Erlichman in their feeding solution. Vogelaar, J. P. M., and Erlichman, E., *Am. J. Cancer*, 1933, **18**, 28.

‡ When horse plasma is used for making the coagulum, it is necessary to add some chicken serum to the medium to obtain growth.