cient amounts they were capable of immunizing guinea pigs. For experiments in concentration such suspensions were cleared of gross material by low-speed centrifugation and then run one and a half hours in a quantity ultracentrifuge[5] using a field of ca 60,000 g. Samples of the supernatant liquids, which were of high protein content, were reserved for tests of immunizing power; the rest was discarded. The large pellets found after ultracentrifugation were resuspended, and their solutions further purified by repetition of the cycle of low-speed centrifugation and ultracentrifugation.

Ultracentrifugal analytical examination of the final solutions has shown the sharply sedimenting boundaries of a molecular species with a sedimentation constant of the order of 60×10^{-13} cm sec.$^{-1}$ dynes^{-1}. In no instance was there to be seen any trace of the more rapidly sedimenting material that may be the infectious substance.[2]

The immunizing capacities of the supernatant fluids and of the final solutions have been tested by subcutaneous injection into 400-gram guinea pigs of two equal doses at an interval of one week, and by intracerebral injection of 100 to 500 minimal lethal doses of active virus two weeks after the second immunizing injection. Complete immunity has been conferred by small amounts of the final product, whereas the supernatant fluids have been devoid of immunizing capacity. In one experiment, for example, there was survival of three out of four guinea pigs receiving solutions containing a total of 0.2 mg. of protein. Two of these animals gave no reaction to 200 lethal doses of virus, the third became ill but promptly recovered. In another experiment in which the protein content of the protective injections was 0.25 mg each, three out of four animals survived 500 minimal lethal doses of active virus without rise in temperature or clinical manifestations of the disease. All guinea pigs injected with the corresponding supernatant fluids died in less than 72 hours. The results of other experiments have been similar. This work is being continued.

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VITAMIN B_1 REQUIREMENTS OF DIFFERENT STRAINS OF WHITE RATS

FROM the time the International Standard of vitamin B_1 was first adopted and made available, the conversion of Sherman Chase units of B_1 to International units has been a point of difference between laboratories. Conversion factors varying from two up to four or five have been reported. These variations have been tentatively explained on the basis of strain differences with the suspicion frequently that diet and technique might contribute largely to the results.

We have had the opportunity in the past two years of using three different strains of white rats in vitamin B_1 work, and have fed a number of groups on different levels of synthetic crystalline B_1. The results obtained with the three strains on a 2 gamma B_1 per day level, with two strains on a 4 gamma level, and one strain on an 8 gamma level, illustrate differences in three strains in their growth response to the feeding of vitamin B_1:

Strain	2 gamma fed daily Ave. gain in 5 weeks Gms.	4 gamma fed daily Ave. gain in 5 weeks Gms.	8 gamma fed daily Ave. gain in 5 weeks Gms.
A ...	33.3 ±1.9	52.8 ±2.3	....
B ...	14 ±1.64	27.6 ±1.04	52.9 ±2.8
C ...	29.8 ±3.38	....	....

These strain differences are inherent, as the young of the breeding stock of these three strains fed on the same stock ration give the characteristic response to B_1 supplements indicated in the table.

Variations in the factor for the conversion of Sherman Chase units to International units can be adequately explained by strain differences in the requirements of the test animals. It is obvious that each laboratory must determine its conversion factor for its particular strain of animals. It is suggested that the development of strains having uniform B_1 requirements is necessary if accurate results are to be obtained.

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THE EFFECT OF HUMIDITY ON THE DEVELOPMENTAL RATE OF CHICK EMBRYOS INCUBATED UNDER INCREASED ATMOSPHERIC PRESSURE

USING a slightly modified pressure incubator, originally described in SCIENCE,[1] a study was made of the effect of humidity on developmental rate of chick embryos during the first eleven days of incubation. Previous studies[2] had shown an acceleration of growth

[5] R. W. G. Wyckoff and J. B. Lagsdin, *Rev. Sci. Instruments*, 8: 74, 427, 1937.

[1] SCIENCE, 80: 99–100, 1934.
[2] *Jour. Elisha Mitchell Sci. Soc.*, 52: 269–273, 1936.

under pressure as compared to controls, the temperature and relative humidity being constant and comparable in both pressure and control incubators. It was noticed, however, that the control eggs lost water more rapidly than the experimental, and it was therefore suggested that the retention of water might be responsible in part for the accelerated growth. Other workers had already observed that increased humidity, within rather wide limits, increased the growth rates of chick embryos at normal pressures.

To determine whether or not the decreased water loss, which in turn is dependent upon the humidity of the surrounding atmosphere, was in part responsible for the increased growth rate of the chick embryos, the humidity in the pressure incubator was lowered until the water loss of the experimental eggs was comparable to that of the controls. When these adjustments had been made, 40 eggs were placed in the pressure incubator and maintained at a pressure of 25 to 30 pounds; 33 control eggs, matched by weight with the experimentals, were incubated at normal pressure. Both lots of eggs were incubated at 100° F.

After eleven days, the embryos were removed, stripped of their membranes and weighed with the following results.

TABLE 1

	Control	Experimental
Average original weight of eggs ..	58.9 gr.	59.7 gr.
Average water loss	1.8 "	2.0 "
Average weight of embryos	3.69 "	6.07 "

Per cent. increase in weight of experimental embryos over the controls was slightly more than 60 per cent.

When compared to the 42 per cent. increase secured in the best of the earlier experiments where the relative humidity in control and experimental incubators was kept the same, it would appear that the retention of water prevents the maximum effect of the pressure, and pressure may now be definitely considered as responsible for the accelerated growth.

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CRYSTALLIZATION OF TOBACCO-MOSAIC VIRUS PROTEIN

METHODS for the crystallization of tobacco-mosaic virus protein specify a preliminary precipitation of certain proteins in amorphous form previous to obtaining the crystalline or liquid crystalline protein. Bawden and Pirie[1] reported that heating the crude infectious sap to 70 C. before clarifying it by centrifugation saved considerable time in that the virus protein could be isolated by direct precipitation with acid or ammonium sulfate, thus eliminating preliminary precipitation with alcohol. It has been found and herein reported that upon heating the macerated frozen tissue to 40 C. and adjusting the reaction to about pH 7.0 by dissolving disodium phosphate salt, and clarifying the juice by filtration, crystallization of the protein comes about during the gradual change of reaction from about pH 7.0 to 6.0 and cooling of the filtrate to room temperature. By adjusting the reaction to pH 4.5 (green to bromecresolgreen) with acetic or sulfuric acid, salting out with 0.3 saturation of ammonium sulfate and storage at 0 to 5 C. over night, further crystallization comes about.

Initial crystallization of the virus protein was obtained in the crude infectious tobacco juice by heating the frozen macerated green tissue to 40 C. for 10 minutes in presence of disodium phosphate salt (35.8 grams per 1,000 grams of green tissue); pressing the liquid through doubled cheesecloth by wringing with the hands; filtering this liquid through celite (25 grams per 1 liter of liquid); allowing the reaction of the filtrate to change without further treatment for 30 minutes (from about pH 7.0 to pH 6.0); adjusting the reaction to pH 4.5 with acetic or sulfuric acid; and dissolving ammonium sulfate to 0.3 saturation (175 gms per liter). Upon agitation the solution possessed a velvety appearance (sheen) which is considered characteristic of crystalline protein in suspension. The crystals developed rapidly and were similar to those illustrated by Stanley.[2] The material was allowed to stand over night at 0 to 5 C. before separation of the crystals from the liquid and subsequent recrystallization by three procedures:

Procedure A. Filtration and dissolving the crystals in alkaline phosphate buffer at pH 8.0 and 40 C. A 1,000 mls sample of the material (first crystallization) was filtered through 25 grams of celite by suction. The residue was dissolved in 500 mls of 0.1 molar phosphate buffer at pH 8.0 and 40 C. and the celite removed by filtration through paper. The filtrate was adjusted to a reaction of pH 4.5 and ammonium sulfate added to 0.3 saturation as in the first crystallization. The procedure was repeated for the third crystallization.

Procedure B. Filtration and Stanley's[3] calcium oxide method for dissolving the crystals. A 1,000 mls sample of the material was filtered as in procedure A,

[1] F. C. Bawden and N. W. Pirie, *Proc. Royal Soc. London*, Ser. B, 123: 274–320, 1937.
[2] W. M. Stanley, *Phytopath.*, 26: 305–320, 1936.
[3] W. M. Stanley, *Jour. Biol. Chem.*, 115: 673–678, 1936.