The results of experiment 1 show that a daily dosage of .2 mgm of iron and .02 mgm of copper promoted a more rapid regeneration of hemoglobin in those rats receiving the smaller intake of milk solids. In only 2 weeks of feeding a difference in recovery concentration of almost 2 grams of hemoglobin per 100 cc of blood developed. In all six pairs the rat on restricted intake recovered the more rapidly, and the probability[3] that this is a fortuitous result is so small (.0033) that it may be neglected. However, in experiment 2, with a daily supplement of .5 mgm of iron and .05 mgm of copper, the result is indecisive, even after 4 weeks of feeding. In 5 of the 7 pairs the rat on restricted food recovered from its anemic condition more rapidly than its pair mate, but in 2 pairs the reverse was true; the statistical analysis (P = .16) indicates that the outcome may have been a fortuitous one. These two experiments on the effect of a variable intake of milk solids are quite analogous to the experiments of Smith and Otis on the comparison of male and female rats in the sense that significant differences were noted only when the supplements were such as to promote a submaximal rate of recovery. They are also in agreement with the theory previously proposed[4] by one of us concerning unbalanced rations, that "the more of them is consumed the poorer nourished will be the animal with reference to the functions with respect to which the rations are unbalanced." Confirmation of this theory has already[5] been reported with rachitogenic diets: the greater the rate of consumption of such diets, the more rapidly does rickets develop. Vitamin B_1-deficient diets are also the more toxic the more of them is consumed,[6] as are also diets deficient in vitamin C.[7]

Experiments 3 and 4 show in both cases that for the particular iron and copper concentrations incorporated in the milk solids, the rats on restricted intake recovered significantly more rapidly than the rats on one half again as much food. It may be said that the concentration of iron used in this experiment (.008 per cent.) was lower than that consumed by the restricted rats in experiment 2 (.010 per cent.), but higher than that consumed by the unrestricted rats (.0067 per cent.).

Unfortunately we have not performed a curative experiment with pairs of male and female rats receiving equal intakes of milk solids. However, the rate of development of anemia in paired male and female rats receiving equal intakes of fresh milk was studied in experiment 5. In feeding periods lasting from 4 to 6 weeks, no significant differences were obtained, although in 4 of the 6 pairs the female rat was the slower in developing an anemic condition. The probability of a chance outcome, .085, is however too large to disregard. It will be noted also that on equal intakes of food the female rats gained less in body weight (P = .046), and from available information it may be assumed that their gains contained less of protein, more of fat and less of blood.

It may be concluded that the sex difference in the development of nutritional anemia noted by H. S. Mitchell, as well as that in the recovery from nutritional anemia noted by Smith and Otis, may be partially or entirely the result of a greater intake of the anemogenic basal diet by male rats. To that extent it is merely a sequel of the well-established difference in growth impulse between the male and the female sex. In the same manner, the frequently observed difference between male and female rats in the rate of calcium retention and of the calcification of the bones has been traced in this laboratory[8] to the greater demand for, and consumption of, food by the male.

The control of food intake by comparative animals in nutrition experiments according to some scheme adapted to the problem at hand will generally simplify their interpretation and will make possible a demonstration of a fact or a principle where lack of control can at best establish only a variable degree of probability in favor of it.

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[4] H. H. Mitchell, SCIENCE, 80: 558, 1934.
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CRYSTALLINE CATALASE[1]

WE have prepared crystalline catalase from beef liver. Our method consists essentially in extracting chopped liver wth dilute dioxane, adding more dioxane to the extract to precipitate impurities and then precipitating the enzyme through the addition of still more dioxane. The precipitated enzyme is dissolved in water and crystallizes upon adding ammonium sulfate and cooling. Crystalline catalase has been obtained also from the extracted liver residue by fractionating extracts with ammonium sulfate solution.

Our catalase crystals are slender plates of microscopic size. Presence of the crystals can be observed by rotating the liquid in which they are suspended and

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observing the thryxotropy. The crystals stain with methyl violet and are fairly soluble in water. Their dilute solution is yellow. Their concentrated solution is brown and gives an absorption band in the red at 627 m μ and a fainter band in the green at 536 m μ.

Catalase can be recrystallized easily by dissolving the crystals in dilute phosphate buffer of pH 7.3, bringing the pH of the solution to approximately pH 5.4 through the addition of acid potassium phosphate and then adding ammonium sulfate slowly with cooling. The crystals form very rapidly.

One sample of twice recrystallized catalase, after dissolving in phosphate buffer and dialysing until free from ammonium sulfate, was found to possess a *"Kat. f"* of 43,000 and an iron content of approximately 0.10 per cent. Crystalline catalase coagulates upon heating and gives many of the usual protein tests. A strong odor of burnt hair is produced on ashing. The pyridine hemochromogen test is readily obtained.

The properties of our crystalline catalase are in complete agreement with the properties of the catalase preparations of von Euler and Josephson,[2] and Zeile and Hellström.[3]

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE USE OF SYNTHETIC RESINS IN THE PREPARATION OF PERMANENT BACTERIAL MOUNTS

THE most common method employed in making permanent preparations of bacterial mounts entails the use of Canada balsam, a naturally occurring resinous substance. It serves as a cementing agent and, when spread upon a glass slide, seals the thin cover slip placed over the mount to protect and preserve the latter for subsequent observation. When permanent mounts are prepared in this manner, several days may be required before the solvent has completely evaporated, leaving a hard cement. In recent years, several synthetic resins have been prepared commercially, solutions of which harden rapidly on exposure to air, giving a hard, clear, colorless layer possessing a refractive index very close to that of glass. Coatings prepared by these resins adhere tenaciously to glass surfaces.

In determining the applicability of these resins, bacterial smears were made on glass slides and stained with dyes commonly used, including crystal violet, carbol fuchsin, methylene blue and the Gram stain. Preliminary investigations indicated that butyl acetate, free of acetic acid, was the most desirable of the organic solvents used. With one resin ("Pontalite"), xylol was substituted for butyl acetate with success. The solutions employed were between 15 and 20 per cent.

Mounts were made in two ways. First, solutions of the synthetic resins were substituted for Canada balsam as the cementing medium. The resin was used in exactly the same manner as the balsam, that is, a drop or two of the solution was placed upon the smear and a glass cover slip placed over it, care being exercised not to include air bubbles. The cover glass was pressed down lightly and the preparation ready for use after ten minutes of air drying.

Secondly, a solution of the resin was applied by tilting the glass slide bearing the mount, lengthwise, at a 45° angle, flooding by means of a dropping pipette and permitting the excess solution to drain off, thus leaving a thin, smooth, glass-like layer of uniform thickness. This may air dry for thirty minutes or more, before being used for observation or, if required at once, drying can be forced by baking the slide at 135° C. for five minutes with no apparent damage to the mount. No cover slip is used, the thin film of resin serving in its stead. When observing mounts prepared in this manner with the aid of the oil immersion objective, xylol can not be used to remove the cedar oil from the slide because of its solvent effect on the resin. The oil can be removed by washing off with ligroin, gasoline or mechanically by merely wiping off with lens paper. Mineral oil, which is often employed as the immersion medium, is much easier to remove from the slides than is cedar oil. If used a great deal, the thin resin layer will eventually become scratched. These scratches can be erased by covering with another film of the resin.

Slides were prepared in February, 1936, in the manner indicated above, using two commercial resins.[1] Each slide was divided into four portions consisting of mounts covered with (a) Canada balsam and a cover slip, (b) the synthetic resin and a cover slip, (c) the synthetic resin alone and (d) an uncovered portion. Over an eleven-month period there has been no noticeable change, such as fading due to the solvent action of the butyl acetate, in any of the covered preparations. Those under the synthetic resin appear as stable as those preserved under Canada balsam.

[2] H. von Euler and K. Josephson, *Liebig's Ann.*, 452: 158, 1927.

[3] K. Zeile and H. Hellström, *Zeit. physiol. Chem.*, 192: 171, 1930.

[1] "Vinylite" (Series A Resin), Carbide and Carbon Chemicals Corporation, New York; "Pontalite," du Pont de Nemours and Company, Wilmington, Del.