bonic anhydrase in crude extracts, and 75% inhibition in dialyzed extracts by 10^{-3} *M* KCN. We believe that plant carbonic anhydrase contributes to, but is not entirely responsible for, the cyanide-sensitivity of photosynthesis in land plants. A detailed presentation of these and related investigations of carbonic anhydrase in plants appeared in Section C, *Canadian Journal of Research.* (C 28, 673 [1950]).

References

1. STEEMANN NEILSON, E., and KRISTIANSEN, J. *Physiologia Plantarum*, **2**, 325 (1949).
2. DAY, R., and FRANKLIN, J. *Science*, **104**, 363 (1946).
3. BRADFIELD, J. R. G. *Nature*, **159**, 3467 (1947).
4. NEISH, A. C. *Biochem. J.*, **33**, 300 (1939).
5. MELDRUM, N. U., and ROUGHTON, F. J. W. *J. Physiol.*, **80**, 113 (1933).
6. BURR, G. O. *Proc. Roy. Soc.* (*London*), **B**, **120**, 42 (1936).
7. BRIGGS, G. E. *Proc. Roy. Soc.* (*London*), **B**, **91**, 249 (1920).
8. CLENDENNING, K. A., and GORHAM, P. R. *Can. J. Research*, **C**, **28**, 114 (1950).
9. HILL, R., and SCARISBRICK, R. *Proc. Roy. Soc.* (*London*), **B**, **129**, 238 (1940).
10. RUBEN, S., KAMEN, M. D., and HASSID, W. Z. *J. Am. Chem. Soc.*, **62**, 3443 (1940).

Animal Protein Factor for the Rat Present in Crude Casein and its Relationships with Vitamin B_{12}[1,2]

M. Piccioni, A. Rabbi, and G. Moruzzi

Istituto di Biochimica, Università di Bologna, Italy

The existence of unknown factors essential to the nutrition of many organisms and present in small amounts in animal protein, and consequently generally denominated animal protein factors (A.P.F.), has been substantiated by several researches, mainly in the field of poultry science. Cary and Hartman *et al.* in 1946 (*1*) reported that an unidentified factor, soluble in hot alcohol (*X* factor), and found in crude casein and liver extracts, is required by the rat for growth, reproduction, and lactation. Further studies (*2*) presented evidence that factor *X* is present even in certain leafy foods. Shortly thereafter, Zucker and coworkers (*3*) reported that a new factor, present in animal protein, and absent from vegetables, soluble in water, dilute acid, alkali, and alcohol-insoluble, is indispensable for rats. This factor was named "zoopherin." Zucker and Zucker (*3*), feeding rats on a complete, purified diet, devoid of zoopherin, observed a high mortality in the newborn, resulting from hemorrhagic lesions in the upper part of the stomach.

For two years we have investigated the effects on rats of a factor present in crude casein. The observations collected, the lesions noted, the kind of diet used, and relationship of the factor to vitamin B_{12} lead us to publish the results.

[1] We wish to thank Cesare Barbieri, of the American Committee, University of Bologna, New York, for having supplied us with the vitamin B_{12}, a product of the American Roland Corporation, used in this investigation.

[2] We acknowledge the assistance of M. A. Dina, of the Pathological Anatomy Institute of the University of Bologna, in the histological examinations of the lesions described.

Rats of our strain were fed the Randoin and Causeret (*4*) diet, which is not a synthetic diet, but rather a natural and extremely varied nourishment. This diet consists of:

	Percentage
Ground cereals (wheat, maize, barley, oats, rye)	88
Wheat germ	5
Crude casein	5
Wheat germ	1.5
Crude casein	0.5

Twice a week the animals also received carrots and vegetables *ad lib.* (salad, lettuce, cabbage, cress, broccoli) and dry yeast. With such a diet Randoin (*4*) and one of us (*5*) have observed optimal growth, reproduction, and lactation for several generations.

Crude casein was the only source of animal protein in this diet. Experiments were then carried out replacing crude with purified casein, made as follows. Casein was suspended in water containing 0.5% acetic acid and a few drops of chloroform. The ingredients were thoroughly mixed, and after slow decantation the liquid was discarded and replaced with new water. The procedure was repeated three times a day for 2 weeks. Finally, the casein was centrifuged down and desiccated *in vacuo*. The animals were kept in special cages in order to avoid possible coprophagy.

A first group of 25 female rats fed a Randoin diet containing purified casein exhibited normal growth, as well as regular reproduction and lactation. The young born of such females (1st generation) exhibited high mortality: 89 out of 126 young rats died (70.6%). The surviving rats reached an almost normal growth: 7 females were able to reproduce. Absolute mortality (100%) was shown by the young of these females (2nd generation). Death did not occur during the first few days of life, but usually between

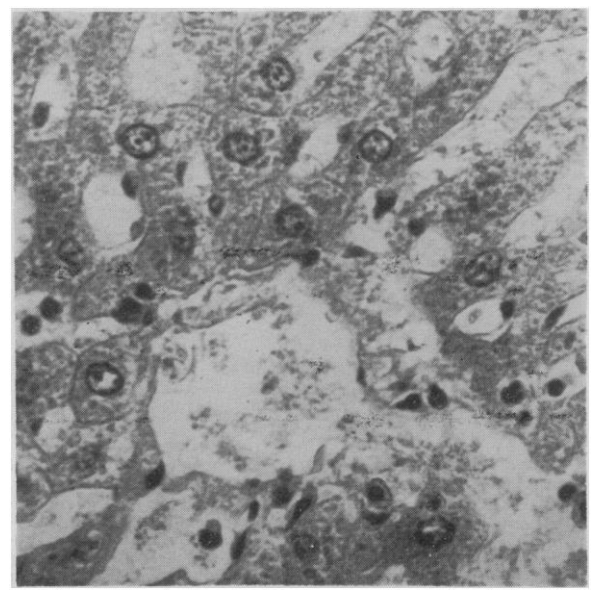

FIG. 1. Normal liver (×720).

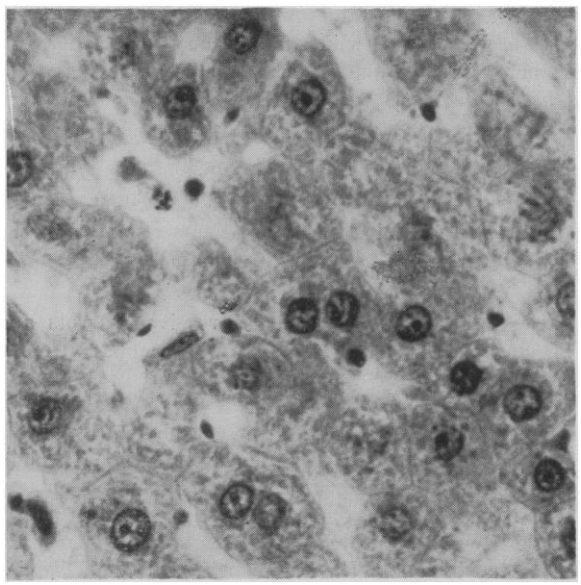

FIG. 2. Liver of rat fed a diet containing purified casein (× 720). Hepatic cells show protoplasmic swelling with granular aspect. Nuclear polymorphism showing some karyorexis. Increase of binuclear cells; increase of Kupffer cells, and limphomonocytic proliferation.

the 15th and 20th days and only rarely after weaning. It is emphasized that the reproductive ability of these females is generally below normal. In addition, the average weight of the newborn of females on a deficient diet is always below normal (av, 3 g instead of 4.4).

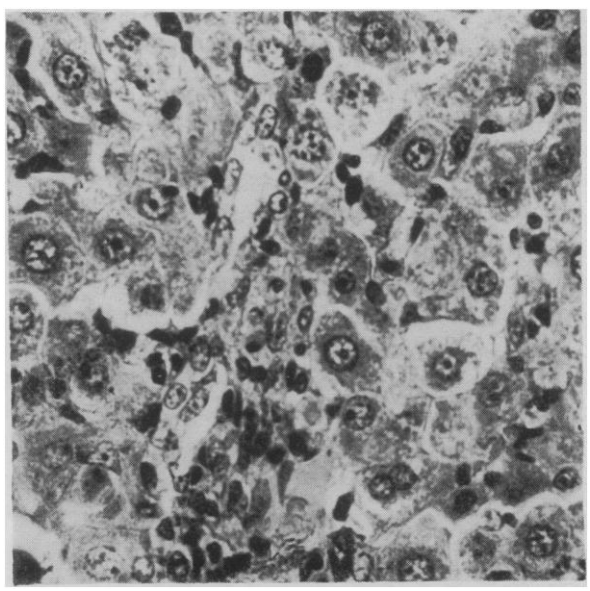

FIG. 3. Liver of rat of the 1st generation (× 720). Diffused protoplasmic swelling of granular and microvacuolar type, with lysis of the hepatic cells and necrobiosis of some isolated elements. Notable nuclear polymorphism, with karyorexis, karyolysis, and chromatin dispersion in the cell. Frequent regenerative phenomena with binuclear cells; irregular hyperchromic nuclei. Enlargement of Kupffer cells, with an intense lymphomonocytary proliferation having the aspect of interstitial hepatitis.

The principal and most serious lesions exhibited by the animals of the 1st and 2nd generations, which probably caused death, were found in the liver, which always showed a very pale color.

Histological preparations of the liver of rats fed a diet containing purified casein (Fig. 2), as well as of the newborn of the 1st (Fig. 3) and 2nd (Fig. 4) generations, are shown in comparison to normal (Fig. 1).

The relationships found by Ott, Rickes, and Wood (*6*) between the animal protein factor of the chick and vitamin B_{12} led us to test the effects of vitamin B_{12} on the newborn of the 2nd generation, in which the hepatic lesions were more evident. Vitamin B_{12}, administered directly in doses of 0.5–5 μg daily,

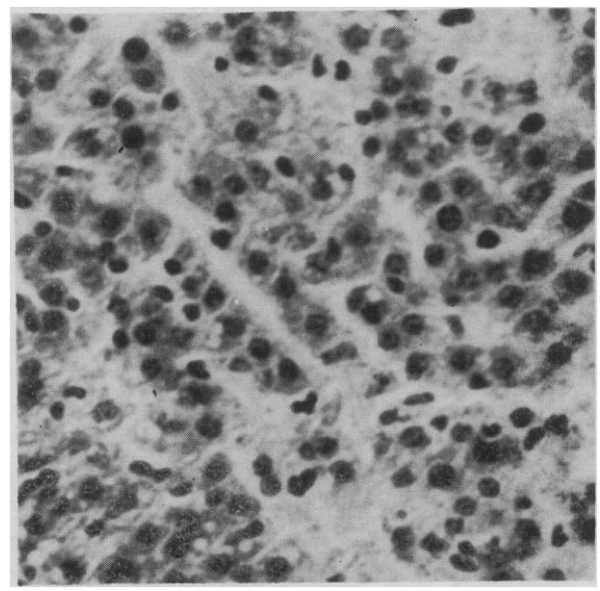

FIG. 4. Liver of newborn of the 2nd generation (× 440). Strong generalized vacuolization of the hepatic cells, with necrotic and lytic zones; absence of mesenchymal reaction; noticeable nuclear polymorphism.

proved to be completely ineffective, whereas small quantities of whole cow's milk saved the animals.

Crude casein contains, therefore, a factor (or factors) indispensable for the normal growth and reproduction of rats. This factor is water-soluble, heat-stable, and scarcely oxidizable, since the crude casein was dried at 100° C in the presence of air. This factor does not seem to be identifiable with vitamin B_{12}. The factor is stored in the organism, and can be exhausted in 2 generations. A deficiency of it causes a high mortality, 70% in the 1st generation and 100% in the 2nd generation.

Death always occurs for whole litters and in a very short time, following an all-or-none principle. The particular histological aspects, and the composition of the diet used, lead us to conclude that the liver lesions described are not related to choline deficiency. In addition we have never observed the gastric hemorrhagic lesions observed previously by others (*3*).

According to our observations, we assume that more than one factor essential for the rat is present in animal proteins.

References

1. CARY, C. A., *et al. Federation Proc.*, **5**, 128 (1946).
2. HARTMAN, A. M., and CARY, C. A. *Federation Proc.*, **5**, 137 (1946).
3. ZUCKER, L. M., *et al. Arch. Biochem.*, **16**, 115 (1948).
4. RANDOIN, L., and CAUSERET, J. *Bull. soc. sci. hyg. aliment.*, **1–3**, 14 (1947).
5. PICCIONI, M. *Boll. soc. ital. biol. sper.*, **26**, 614 (1950).
6. OTT, W. H., RICKES, E. L., WOOD, T. R. *J. Biol. Chem.*, **174**, 1047 (1948).

A Micro Ball Mill for the Disintegration of Bacteria

Carl-Göran Hedén

The Cell Research Department,
Nobel Institute for Medical Research,
Stockholm, Sweden

For grinding small quantities of bacteria a centrifuge tube with a ground-in glass rod can be quite satisfactory, but it is difficult to obtain the same mechanical effects if a number of specimens must be treated simultaneously. For this reason the vibrator equipment described below was designed.

The use of 50 cps vibrations for disintegrating biological materials has been described by E. Hammarsten (*1*). The present author found the method very useful and devised an extension of the technique, which may also prove of value for grinding other biological materials besides bacteria.

The grinding was effected in glass tubes made out of ordinary pyrex glass tubing (diam, 7 mm), which was cut about 15 cm long. The pieces were bent in the middle to resemble the tube in Fig. 1. A special holder made out of brass and containing Woods metal (Fig. 2) was heated to melt the alloy (mp 65.5° C), and the tubes were then inserted in a row. When the metal had solidified, the tubes were taken out of their setting and the material in question—mostly in the form of a freeze-dried powder—was added. Up to 42 specimens could be accommodated. Nine carefully chosen glass beads of a diameter slightly smaller than that of the tube were also added to each specimen, and the tubes were plugged with rubber stoppers. They were replaced in their settings, and a brass pole covered with a piece of rubber hose was placed in the angle of the tubes and screwed tightly down so as to keep them firmly in place. The holder was mounted on the stage of a 50 cps a.c. vibrator (Type EMR6, 70 w, 200 v, Vibroverken Ltd., Stockholm) in a freezing box at −15° C. The vibrations were mainly vertical and caused the glass beads to be thrown between the walls of the tube, but owing to the slope of the branches they also developed a rotating movement (arrows, Fig. 1), which counteracted the tendency of the material to accumulate at the bottom of the tube and effected a continuous mixing of the material. Treatment for 4 hr was not accompanied by any perceptible rise in temperature and was as a rule sufficient to break up the bacteria. The treatment could be shortened by adding weighted amounts of glass powder to the specimens or by etching the glass beads and the inside of the tubes with fluorine.

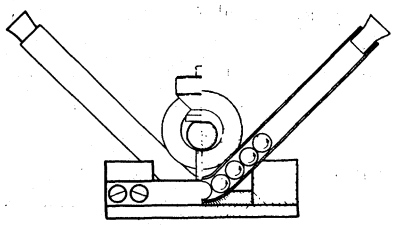

FIG. 1.

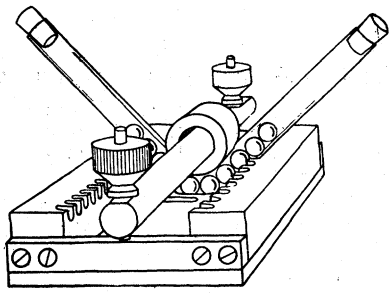

FIG. 2.

Reference

1. HAMMARSTEN, E. *Acta Med. Scand.* Suppl. 196, 634 (1947).

The Purine and Pyrimidine Content of Three Strains of *Escherichia coli*[1]

A. Marshak

New York University College of Medicine and *Division of Tuberculosis, USPHS*

Witkin isolated a spontaneous mutant, B/r, of *E. coli* strain B which proved to be more resistant than the parent to both x-rays and ultraviolet light (*1*). These two strains, together with strain K_{12} which was found by Lederberger to show genetic recombination unusual in *E. coli* (*2*), have been analyzed for purine and pyrimidine content.

The organisms were grown in a synthetic medium, with ammonium chloride as source of nitrogen and with succinate and glutamate as source of carbon. In each case 24-hr cultures were harvested by centrifugation, and the organisms extracted with cold 7% trichloracetic acid, ethanol, and boiling ethanol-ether. The residue after being dried *in vacuo* was rendered to a powder. Approximately 10 mg of the powder was digested with $HClO_4$, the digest subjected to chromatography, and the yield of nitrogenous bases

[1] Supported by a grant from the Research Grants Division of the USPHS.