

The effect of bovine γ -globulin concentration is shown in Table 3. Fluids and extracts were harvested two days after planting.

K. M. STEVENS
J. M. MCKENNA

Department of Virology,
Merck Sharp and Dohme Research Laboratories,
West Point, Pennsylvania. Dec. 19.

¹ Steiner, D. F., and Anker, H. S., *Proc. U.S. Nat. Acad. Sci.*, **42**, 580 (1956).

² Carrel, A., and Ingebrigtsen, R., *J. Exp. Med.*, **15**, 287 (1912).

³ Parker, R. C., *Science*, **85**, 292 (1937).

⁴ Salle, A. J., and McOmie, W. A., *J. Immunol.*, **32**, 157 (1937).

⁵ Kimura, R., "Tissue Culture" (Ejnar Munksgaard, Copenhagen, 1953).

⁶ Johnson, A. G., Gaines, S., and Landy, M., *J. Exp. Med.*, **103**, 225 (1956).

⁷ McKenna, J. M., and Stevens, K. M., *J. Immunol.* (in the press).

⁸ Dunn, M. S., "Biological Preparations", **1**, 22 (John Wiley and Sons, New York, 1950).

⁹ Hanks, J. H., and Wallace, R. E., *Proc. Soc. Exp. Biol. Med.*, **71**, 196 (1949).

¹⁰ Boyden, S. V., *J. Exp. Med.*, **93**, 107 (1951).

Antagonism of γ -Butyrobetaine and Carnitine on Chick Embryonic Bones cultivated *in vitro*

CARNITINE (β -hydroxy- γ -butyrobetaine) stimulates the growth and the periosteal ossification of embryonic chick tibias and femurs cultivated *in vitro*^{1,2} using the watch-glass technique³. This effect can be demonstrated if the embryo extract of the medium is diluted 20 or 200 times in Tyrode solution to get rid so far as possible of the natural carnitine always present in it (44–88 $\mu\text{gm./gm.}$ dry weight for a 12-day-old chick embryo⁴). Measurements of the thickness of periosteal bone and counts of mitoses had indicated that the periosteum is a very sensitive site for the action of carnitine.

Fraenkel and his collaborators⁵ observed that γ -butyrobetaine in large doses antagonizes the effect of carnitine in the survival of *Tenebrio molitor* fed a synthetic diet.

We have tried to demonstrate the antagonism between γ -butyrobetaine and carnitine on embryonic bones cultivated *in vitro* under the conditions previously described^{1,2}.

Tibias and femurs of 7-day-old chick embryos were cultivated for seven days. Controls were prepared with bones of the opposite side of each embryo. Histological sections were stained by the azan method after fixation in acetic Zenker's fluid. As in previous experiments, carnitine was added as 'dicarnitine', a synthesized compound ('Bicarnesine' Labaz)⁶ having the same biological properties as carnitine according to Leclercq's results⁷ with *T. molitor*. γ -Butyrobetaine was kindly supplied by Dr. G. Fraenkel.

In the first set of experiments, 100–2,000 $\mu\text{gm.}$ of γ -butyrobetaine was added per ml. of culture medium made of equal parts of fowl plasma and 20-fold diluted embryo extract; the pH was next adjusted to neutrality. 1,000 $\mu\text{gm.}$ or more γ -butyrobetaine then inhibited growth and induced pycnosis of all nuclei. The addition of 5 $\mu\text{gm.}$ of dicarnitine to the medium containing 1,000 $\mu\text{gm.}$ of γ -butyrobetaine restored normal growth (in comparison with controls) and practically prevented pycnosis. The growth inhibition and pycnosis produced by 2,000 $\mu\text{gm.}$ γ -butyrobetaine could not be counteracted by 5 $\mu\text{gm.}$ of dicarnitine.

Table 1

γ -Butyrobetaine ($\mu\text{gm./ml.}$)	Dicarnitine ($\mu\text{gm./ml.}$)	Inhibition of growth	Pycnosis in other cells	
1. With 20-fold diluted embryo extract :				
100-500	0	—	—	—
1,000	0	+	+	+
1,000	5	—	—	—
2,000	0	+	+	+
2,000	5	—	few	few
2. With undiluted embryo extract :				
1,000	0	—	—	—
2,000	0	?	+	—
2,000	5	—	—*	—
4,000	0	+	+	few
4,000	5	—	—†	—

* No pycnosis in 8 cases out of 10.

† No pycnosis in 2 cases out of 4.

In the second set of experiments undiluted embryo extract was mixed with plasma as in the original method of Fell and Robison⁸. A concentration of 1,000 $\mu\text{gm.}$ of γ -butyrobetaine had no inhibitory action on growth and the tissues remained healthy; 4,000 $\mu\text{gm.}$ had a slight but regular inhibitory effect on the growth-rate. Irregularly distributed areas of pycnotic nuclei were observed in all histological sections with a dose of 2,000 $\mu\text{gm./ml.}$ or more; all cells damaged were osteoblasts. The addition of 5 $\mu\text{gm.}$ of dicarnitine suppressed pycnosis among osteoblasts in eight cases out of ten. Healthy tissues were sometimes obtained even when 4,000 $\mu\text{gm.}$ of γ -butyrobetaine had been added per ml. of medium together with 5 $\mu\text{gm.}$ of dicarnitine (two cases out of four). These results are summarized in Table 1.

Fraenkel and his collaborators⁵ considered the possibility that γ -butyrobetaine "is acting not to form carnitine but to displace it from its usual site of action". Our experiments show such an effect in the periosteal osteoblasts of embryonic bone cultivated *in vitro*. These cells seem selectively sensitive to the effects of both carnitine and γ -butyrobetaine. Our results are in agreement with the recent report by Ito and Fraenkel⁹, who showed a similar antagonism on chick blastoderms.

A detailed account of our findings will appear in the *Archives de Biologie*.

SUZANNE LIÉBECQ-HUTTER
Z. M. BACQ

Institut d'Histologie et
Laboratoire de Pathologie et
Thérapeutique Générales,
University, Liège. Jan. 24.

¹ Liébecq-Hutter, S., *Voeding*, **16**, 791 (1955).

² Liébecq-Hutter, S., *J. Embryol. Exp. Morph.*, **4**, 279 (1956).

³ Fell, H. B., and Robison, R., *Biochem. J.*, **23**, 767 (1929).

⁴ Fraenkel, G., *Biol. Bull.*, **104**, 359 (1953).

⁵ Bhattacharyya, P. K., Friedman, S., and Fraenkel, G., *Arch. Biochem. Biophys.*, **54**, 424 (1955).

⁶ Dechamps, G., Buu-Hoi, N. P., Le Bihan, H., and Binon, F., *C.R. Acad. Sci., Paris*, **238**, 826 (1954).

⁷ Leclercq, J., *Arch. Int. Physiol.*, **62**, 101 (1954).

⁸ Ito, T., and Fraenkel, G., *Fed. Proc.*, **15**, 558 (1956).

Effects of Cortisone Acetate upon the Distribution and Excretion of Radioyttrium

HORMONE treatments have influenced to varying degrees the distribution and excretion of heavy radioactive metals in animals. Parathormone altered radiostrontium distribution¹, but it had no apparent effect on internally deposited radioisotopes of cerium, plutonium or yttrium^{2,3}. Adrenocorticotrophic hor-