firms that it acts extracellularly. The predominant site of uptake of C-10 and binding of curare is most likely the motor end-plate, as shown by autoradiography (WASER[12,13]; WASER and LÜTHI[14]). Studies on the distribution of C-10-H^3 in sections of diaphragm muscle (TAYLOR, CREESE, NEDERGAARD and CASE[17]) also suggest that C-10-H^3 is taken up through the motor end-plates.

The uptake and phase II block of C-10 appears to be related since their time course is approximately the same. This is supported also by the finding that curare can prevent the onset of phase II block of depolarizers (NEDERGAARD and TAYLOR[18]) and decrease the uptake of C-10-H^3 [19].

Résumé. Le décaméthonium, une substance dépolarisante, est absorbé par le muscle squelettal tandis que la substance non-dépolarisante, le diméthyltubocurarine, ne l'est pas. L'absorption du décaméthonium se rapporte peut-être à ce bloque de deuxième phase qui se fait voir avec les substances dépolarisantes.

O. A. NEDERGAARD and D. B. TAYLOR

Department of Pharmacology and Brain Research Institute, University of California Center for Health Sciences, Los Angeles (California 90024, USA),
April 28, 1966.

[17] D. B. TAYLOR, R. CREESE, O. A. NEDERGAARD, and R. CASE, Nature, Lond. *208*, 901 (1965).
[18] O. A. NEDERGAARD and D. B. TAYLOR, Biochem. Pharmac., Suppl. 12, 165 (1963).
[19] The authors express their gratitude to Dr. R. CREESE, Department of Physiology, University of London, for helpful criticism of this manuscript.

Stability of the Colloidal Chromic Radiophosphate (^{32}P) to the Isotopic Exchange

The relatively high percentage of ^{32}P accumulation observed in bone (especially bone marrow) after the injection of colloidal solutions of chromium phosphate, has been explained by several authors[1,2] as being due to the liberation of phosphate ions in the organism mainly because of the instability of the chemical bond in the chromic phosphate molecule.

This work has been done to test the chromium phosphate stability to the isotopic exchange with the ionic phosphate, normally present in the organic fluids.

Two different types of colloidal chromic phosphate (^{32}P) have been assayed: a true colloidal solution (type B) and one with a larger particle suspension (type F). The latter is currently used as a therapeutic agent.

Type B[3] was prepared by heating at 70–80 °C a mixture of 1.5 ml of H_3PO_4 solution (10 mg/ml), 1.8 ml of CrO_3 solution (10 mg/ml) and the ^{32}P activity (1 mc of carrier-free ^{32}P) in 2 ml of distilled water. Then, stirring continuously, 100 mg of Na_2SO_3 dissolved in 3 ml of 2% gelatin solution were added. After being boiled for a few minutes and then cooled to room temperature, the almost clear blue-green solution was dialysed against distilled water until no activity was detected in the water. The radioactive yield was 40–50%.

Type F[3] was prepared by mixing 4 ml of H_3PO_4 solution (10 mg/ml) with 5 ml of CrO_3 solution (10 mg/ml) and the ^{32}P activity incorporated. After heating for 15 min in a boiling water bath, 2 ml of Na_2SO_3 solution (200 mg/ml) and, immediately, 2 ml of 6% gelatin solution were added. The heating was continued another 10 min and then the excess of ionic phosphate was eliminated by dialysis as described before. The radioactive yield was 75%.

In both preparations, the final concentrations were chromic phosphate 3 mg/ml and gelatin 6 mg/ml.

The isotopic exchange was studied by incubation, at 37 °C, of 10 μC of colloid (tested phosphate ion-free by electrophoresis) with an isotonic phosphate solution at pH 7.2 (1 vol 2.1% KH_2PO_4 + 3 vol 2.2% $Na_2HPO_4 \cdot 2H_2O$). The incubation was carried out under sterile conditions and samples were taken at different intervals: 1, 2 and 6 h; 1 day, 2, 5, 7, 9 and 12 days. The activity as ionic phosphate was determined by electrophoresis using buffer veronal-sodium veronal pH 8.6 for 1 h at a voltage gradient of 15–20 V/cm.

The distance of migration of both ionic phosphate and colloidal chromic radiophosphate was determined by

[1] S. W. ROOT, M. P. TYOR, G. A. ANDREWS, and R. M. KNISELEY, Radiology *63*, 251 (1954).
[2] E. S. KISELEVA and S. L. DARYLOVA, Med. Radiol. (USSR) *9*, 11, 29 (1964).
[3] L. J. A. ANGHILERI, Int. J. appl. Radiat. Isotopes *16*, 623 (1965).

Percentage of ^{32}P as chromic phosphate after incubation

Time	1 h	2 h	6 h	24 h	2 days	5 days	7 days	9 days	12 days
Type B	98.3 ± 0.5[a]	98.3 ± 0.5	98.3 ± 0.5	97.6 ± 0.4	97.9 ± 0.1	97.0 ± 0.2	96.7 ± 0.0	96.6 ± 0.4	96.6 ± 0.4
Type F	95.8 ± 0.2	95.8 ± 0.2	95.8 ± 0.4	94.2 ± 1.4	94.6 ± 1.4	93.5 ± 0.3	93.7 ± 0.7	93.4 ± 0.1	93.4 ± 0.2

[a] Standard deviation.

scanning. Then the electrophoregrams were cut where both peaks of activity were found. After elution with 2 *N* HCl, an aliquot was evaporated on a glass planchet under an IR-lamp and counted with a thin-window Geiger-Müller counter. The self-absorption corrections were made using a self-absorption curve. In this way the % of radioactivity corresponding to each peak was calculated. The Table gives the experimental values for a series of determinations made in triplicate.

The results show a very good stability to the isotopic exchange for both preparations, being a little more stable than true colloidal solutions. Possibly because of the faster large particle formation, a small amount of ionic phosphate (not removable by dialysis) is occluded into the particles. This phenomenon does not occur with the true colloidal solution.

Résumé. Nous avons étudié la stabilité du phosphate (^{32}P) chromique colloidal, par rapport à l'échange isotopique avec le phosphate ionique. Après 12 jours d'incubation à 37 °C les solutions présentent seulement 6,6% de ^{32}P ionic échangé. Les préparations ont montré une très bonne stabilité.

L. J. Anghileri[4] and R. Marqués

Comisión Nacional de Energía Atómica, Buenos Aires (Argentina), January 21, 1966.

[4] Present address: Institut du Radium, Laboratoire Curie, Paris (France).

Effect of Growth Hormone and Prolactin on Mouse Transplantable Mammary Adenocarcinoma

The role played by growth hormone and prolactin in the development and growth of mammary tumours has not yet been established. Early studies using impure preparations showed that continued administration of growth hormone to normal rats, either male or female, accelerated the development of neoplasms in the lung, adrenal medulla and reproductive organs[1,2]. Transplantation into mice of tumours of the anterior pituitary (MtT) has been found to accelerate the rate of appearance of mammary tumours induced by X-ray, virus or 3-methyl cholanthrene[3–5]. Only a few attempts have been made to study the effect of these hormones on mammary adenocarcinoma in mice[6–8]. The results obtained by these investigators differ from one another, possibly because of the use of insufficiently purified hormone preparations.

The present study was undertaken in order to determine the effect of purer preparations of growth hormone and prolactin, which have only recently become available, on the growth of two kinds of transplantable mammary adenocarcinoma in mice.

Inbred strains of R III and C_{57}BL female mice, weighing from 17–18 g, were used. They were fed Purina chow and water ad libitum. MMC_1A and Eo 771 carcinomas, transplanted for over 100 passages in our laboratory, were employed. For the experiment the tumour was implanted subcutaneously into the right axillary region by a sterile trocar (No. 16). The following hormone preparations were used: bovine growth hormone (Choay, Batch S-407B) and sheep prolactin (Ferring, Batch 31209). The hormones were dissolved in saline with the addition of 0.1 *N* NaOH and injected i.p. in a daily dose of 200 or 300 µg/0.2 ml for 10 days starting 24 h after the implantation of the tumour. Matched control groups were injected with the same volume of solvent. The animals were sacrificed on the 12th day after the transplantation. Both the whole animal and the excised tumours were accurately weighed.

It may be seen that both BGH and sheep prolactin produced enhanced tumour growth in MMC_1A as well as

[1] H. D. Moon, A. A. Koneff, C. H. Li, and M. E. Simpson, Proc. Soc. exp. Biol. Med. *93*, 74 (1956).
[2] H. D. Moon, M. E. Simpson, C. H. Li, and H. M. Evans, Cancer Res. *10*, 297, 364, 549 (1950).
[3] K. Jokoro, J. Furth, and N. Haran Ghera, Cancer Res. *21*, 178 (1961).
[4] K. Jokoro and J. Furth, J. natn. Cancer Inst. *29*, 887 (1962).
[5] U. Kim and J. Furth, Proc. Soc. exp. Biol. Med. *103*, 646 (1960).
[6] R. N. MacAlpin, S. M. Blair, D. R. Giles, W. R. Lyons, and C. H. Li, Cancer *8*, 271 (1955).
[7] M. C. Smith, P. A. Slattery, M. B. Shimkin, C. H. Li, R. Lee, J. C. Clarke, and W. R. Lyons, Cancer Res. *12*, 59 (1956).
[8] M. P. Shulman and D. M. Greenberg, Proc. Soc. exp. Biol. Med. *72*, 676 (1949).

Table I. The effect of bovine growth hormone (BGH) and sheep prolactin on the growth of transplanted mammary adenocarcinoma (MMC_1A) in R III female mice

Treatment	No. of mice	Tumour weight mg mean ± S.D.
Saline control	11	1010 ± 489
BGH, 200 µg	14	1525 ± 608
Sheep prolactin, 200 µg	12	1775 ± 400
BGH + prolactin	13	1870 ± 422

Table II. The effect of bovine growth hormone (BGH) and sheep prolactin on the growth of transplanted mammary adenocarcinoma (Eo 771) in C_{57}BL female mice

Treatment	No. of mice	Tumour weight mg mean ± S.D.
Saline control	14	827 ± 420
BGH, 200 µg	15	1485 ± 489
BGH, 300 µg	10	1379 ± 484
Sheep prolactin, 200 µg	13	1183 ± 316