LETTERS TO THE EDITORS

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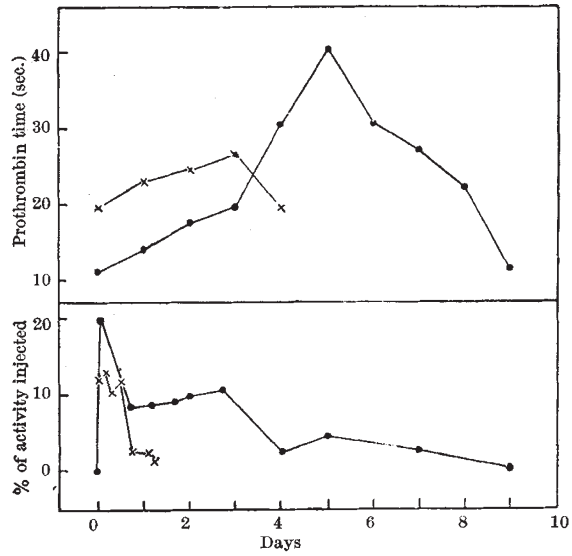
Tracer Experiments in Mammals with Dicumarol Labelled with Carbon-14

DICUMAROL[1] is widely used clinically for the treatment of thrombosis. Since very little is known about the mechanism whereby dicumarol lowers the prothrombin-level in blood[2], it was thought that experiments with dicumarol labelled with carbon-14 might be of value. The dicumarol was prepared by adding an aqueous solution of formaldehyde containing carbon-14, obtained from the Oak Ridge National Laboratory, to a saturated aqueous solution of 4-hydroxycoumarin[3]. The 3,3′-methylene-C^{14}-*bis*(4-hydroxycoumarin) so obtained had a specific activity of 6×10^4 counts per minute per milligram, when measured with an end-window type of Geiger–Müller counter and a scale of 128.

Fourteen mice were each given 0·25 mgm. labelled dicumarol by intravenous injection. At various times after the injection, the animals were sacrificed, the tissues weighed and radioactivity determined. No significant amount of activity could be detected in the lungs, kidney and tissues in general; but large amounts of activity were found in the liver, gall bladder, fæces and urine. 80 per cent of the activity disappeared from the blood in the first hour following injection. A series of rabbits was similarly given 10 mgm. each of labelled dicumarol. A similar distribution of radioactivity was found in these animals with a rapid disappearance of dicumarol from the blood and a rapid increase of activity in the liver. By means of the isotope dilution procedure, it was shown that the activity found in the liver was essentially all unchanged dicumarol. By contrast, although a large percentage of the activity was recovered in the urine at the end of the experiment, none of the activity in the urine was due to dicumarol.



Plasma prothrombin time and radioactivity in liver of mice and rabbits after intravenous injection of dicumarol labelled with carbon-14. ×——×, mice (0·25 mgm. dicumarol); O——O, rabbits (5 mgm./kgm. dicumarol)

The rate of disappearance of dicumarol in the livers of the mice and rabbits is shown in the accompanying graph together with the changes in prothrombin time. It can be seen that, in the mice, the dicumarol remained in the liver for one day and the prothrombin-time remained high for three days, whereas in the rabbits the dicumarol remained in the liver for seven days and the prothrombin-time remained high for eight days. In further experiments, mice were injected with labelled dicumarol together with vitamin K. It was found that, for the animals receiving the vitamin, activity appeared in the liver immediately after the injection; but that in the following hours, the activity disappeared from the liver much more quickly than for the animals not receiving vitamin K.

These results demonstrate that the liver constitutes the target organ for the action of dicumarol, and they suggest that the period of time during which dicumarol remains in the liver is related to the effectiveness of this agent in interfering with the formation of prothrombin.

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[1] Campbell, H. A., and Link, K. P., *J. Biol. Chem.*, **138**, 21 (1941).
[2] Shapiro and Weiner, "Coagulation, Thrombosis and Dicumarol".
[3] Lee, Trevoy, Jaques and Spinks, *Can. J. Res.* (in the press).

Mechanism of Absorption of Inorganic Phosphate from Blood by Tissue Cells

IN the course of a study of the phosphorus metabolism of liver, evidence was obtained suggesting that the absorption of inorganic phosphate ions from the blood is not a simple exchange of ions across the cell membrane but that it requires an energy-yielding phosphorylating mechanism. Accordingly, this hypothesis was tested by experiments in which rabbit liver left *in situ* was perfused with homologous plasma to which a small amount of carrier-free inorganic $^{32}PO_4^{3-}$ was added (2–5 μc. per litre) with or without 0·01 *M* sodium azide. The heparinized plasma, after addition of phosphorus-32 in saline, was divided into two equal volumes to one of which sodium azide was added; the two lots were used for the control and experimental perfusions, which were carried out on the liver of litter-mate animals.

The rabbits were heparinized one hour before the experiment and then were anæsthetized with nembutal and ether. The liver was prepared for the perfusion by inserting a cannula into the portal vein and tying off both the inferior vena cava above the right renal vein and the hepatic artery. A cannula was also tied into the inferior vena cava just above the diaphragm to collect the outflow from the liver. The level of the outflow was 2 cm. above the highest point of the liver, ensuring that all parts of the organ were perfused. Before the perfusion was begun, gentle pressure was applied to the liver and as much blood as possible was drawn from the outflow cannula