one month; they indicate that creatinine at birth is at or near the adult level and falls within about two weeks to the level of the month old infant. Premature infants show serum levels which are higher and rates of excretion which are lower than full term infants of the came postnatal age; this is undoubtedly related to the degree of immaturity of the kidney in these infants.

Conclusions. (1) The technic described measures all of the creatinine and only creatinine in blood and urine. (2) There is a gradual rise in the serum concentration and in the rate of urinary excretion of creatinine from one month of age to puberty, when adult levels are reached.

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Dicumarol Labelled with C^{14}.* (17838)

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As a result of the classical work of Link(1), dicumarol has come into widespread clinical use as a therapeutic agent against thrombosis. However, there has been little information available regarding the mechanism whereby dicumarol lowers the prothrombin level in the blood until the recent demonstration by Lupton(2) that after treatment of rats with dicumarol, the livers fail to add prothrombin to blood perfused through them. In an attempt to obtain further information regarding its action, dicumarol labelled with radioactive C^{14} was used in animal experiments.

Methods. Dicumarol, labelled with C^{14} in the methylene bridge, was synthesized using formaldehyde-C^{14}. The labelled dicumarol was dissolved in 0.1 N sodium hydroxide (5 mg per cc) and injected intravenously in mice and rabbits. 0.05 cc of solution (0.25 mg) was given to each mouse, while each rabbit (weight 2 kg) received 2.0 cc. To determine radioactivity in the blood, samples were taken, weighed, dried and the material spread uniformly in a platinum dish. Other tissues were treated similarly. Radioactivity measurements were then made with the end-window type of Geiger-Muller chamber with a scale of 128 counting unit. Correction for self-absorption of C^{14} activity was made. Prothrombin times were determined by the usual methods using 0.02 M calcium chloride solution.

Results. Fourteen mice were each given 0.25 mg of labelled dicumarol of specific activity 120.4 registers/min./mg intravenously (1 register = 128 counts). The first 4 mice were placed in a metabolism apparatus and the expired CO_2 collected and tested for radioactivity. No radioactivity whatsoever could be determined in the expired air and as a consequence, it was not necessary to collect expired air in further experiments. At various times after the injection, the animals were sacrificed and after weighing, the radioactivity determined for lungs, blood, liver, gall-bladder, kidneys, stomach, intestines, intestinal contents, feces and urine. No significant amount of activity could be detected in the lungs. Results for other tissues are shown in Fig. 1. Large amounts of radioactivity were detectable in the liver, intestines, gall-bladder, feces and urine. A trace was found in the kidney and stomach. Activity was determined separately on the intestine and in-

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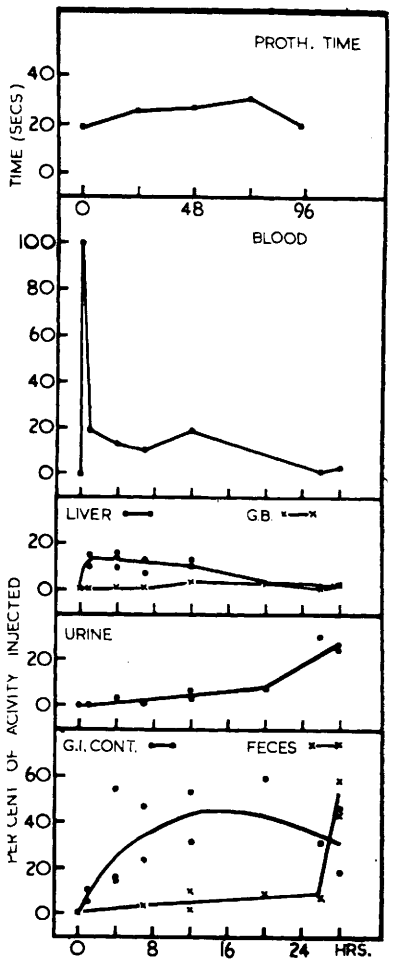

FIG. 1.

Blood and tissue levels of radioactivity in mice following C^{14}-labeled dicumarol. 0.25 mg (= 10 mg/kg) injected intravenously at zero time. Value for urine and feces is total excretion to time shown. G.I. Cont. = Gastro-Intestinal Contents. G.B. = Gall Bladder and Contents.

testinal contents and feces. Since it was found that the value for the intestine was the lower, the more effective the removal of intestinal contents, values for intestine and intestinal contents have been reported together while the true values for the intestine proper are probably quite small. The kidneys contained about 2-4% of the activity until 12 hours following injection. After 18 hours only a trace of activity remained in the kidney. The specific activity was at no time greater than that of the lung and hence the activity found was due to the blood present in the kidney It was of interest to find that the bile showed the highest specific activity per gram of wet tissue, one sample of bile showing twice the initial concentration of radioactivity in the blood. It is to be noted that the values given in Fig. 1 for tissues and intestinal contents show actual values for activity at the given time, whereas urine and feces show total excretion from zero to the given time. Values for urine include the radioactivity found in the urinary bladder. Determinations of the activity of plasma from the blood samples demonstrated that all the activity was in the plasma, none in the cells.

Fig. 1 shows very interesting features in the time relationships of the appearance of the activity in different organs. The activity disappeared rapidly from the blood. After one hour, the total activity was distributed between blood, liver and intestine in decreasing order. From the 1st to the 12th hour, levels of activity in blood and liver remained approximately constant but that in the intestinal contents increased until it represented the major part of the activity. After the 12th hour, the activity in liver and blood fell to a low level. However, appreciable activity was now found in the urine and this increased until by the 28th hour, 30% of the dose had been excreted in the urine. At the termination of the experiment at the 28th hour, a high percentage of the activity had appeared in the feces. Judged by the radioactivity, these results indicate that dicumarol disappeared rapidly from the blood and was taken up by the liver. One hour after the injection, 10-15% of the injected dicumarol was present in the liver. At this time there was very little activity in other tissues. However, while there was little decrease in the level of dicumarol in the liver after four hours, a large amount of the activity was now found in the intestinal contents. The very high specific activity found in the bile suggested that this activity reached the intestine from the liver via the bile ducts. After the fourth hour, activity began to appear in the kidneys and urine, although urinary excretion of activity was not significant until after 12 hours and was greatest between the 18th and the 28th hour. This suggested that

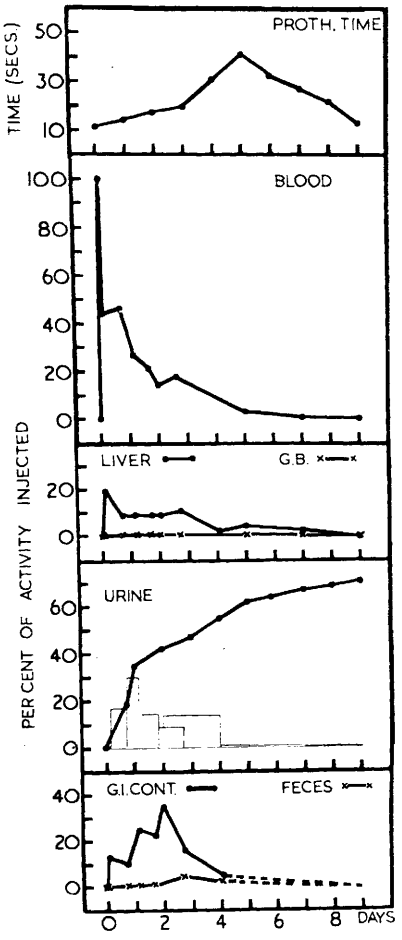

FIG. 2.

Blood and tissue levels of radioactivity in rabbits following C^{14}-labeled dicumarol. 5 mg/kg injected intravenously. For urine, histograms show 24-hour excretion prior to death; continuous line shows total excretion for a single rabbit followed for the whole experiment.

urinary excretion was not due to the injected dicumarol but to activity that had been reabsorbed from the intestinal tract. This was probably also responsible for activity observed in the blood after the 7th hour. At the 28th hour the activity in tissues was practically nil as the result of excretion, chiefly in the feces, although 30% of the activity was found in the urine.

The results for a series of rabbits, similarly injected with radioactive dicumarol, are shown in Fig. 2. Again there was a rapid, though slower, disappearance of dicumarol from the blood. In 4 hours, 30% of the injected dicumarol was found in the blood. The activity of the liver rose rapidly to 20% of the injected activity after 2 hours and then fell to about 10% and remained there for 3 days, at which time the prothrombin time started to rise and radioactivity in the liver fell to about 2% of the dose. The intestinal contents again showed activity, about 12% of the activity being found in them 2 hours after the injection and reaching 25% of the dose after 24 hours. However, in contrast to the mouse, there was practically no significant activity found in the feces even at the termination of the experiment, when the total amount of activity excreted in the feces was less than 5%. Activity was found in the urine. Excretion of 20% of the activity was found in the urine and urinary bladder by the 18th hour and approximately 72% of the total activity was recovered from the urine by the end of the experiment. The kidney showed no higher specific activity than the lung, etc. and the amount in the kidney at no time exceeded 6% of the dose injected. Again, there was considerable activity found in the bile and gall-bladder, this material having the highest specific activity of any tissue. These results were similar to those obtained in the mouse with the exception that no excretion of the activity was found in the feces. Apparently all the activity excreted into the intestine by the liver via the bile was reabsorbed and excreted by the kidneys. It can be observed that the activity remained in the liver much longer for the rabbits than for the mice.

Nature of the activity in the liver. In order to determine whether the radioactivity observed in the liver was due to C^{14} still attached to dicumarol, a rabbit was injected with 10 mg of labelled dicumarol (specific activity, 140 registers/min/mg) and after 24 hours was sacrificed and the liver removed. The liver was comminuted and to it was added 29 mg of inactive dicumarol to provide a carrier. After mixing thoroughly, the activity was extracted from the liver with acetone. Most of the acetone was removed by distillation, sodium hydroxide was added and the mixture extracted three times with ether. The alkaline solution was now acidified with

HCl and the dicumarol extracted with ether. On evaporation of the ether, a crude product was obtained containing all the radioactivity of the original liver. All fractions discarded were checked for radioactivity and no significant activity could be detected in these fractions. The crude dicumarol so obtained was subjected to repeated recrystallizations from cyclohexanone. After 5 recrystallizations, 2.6 mg of product was obtained with a melting point of 288°C, mixed melting point of 288°C, and a specific activity of 4.1 registers/min/mg. If the number of mgs of labelled dicumarol present in the liver (before adding carrier) is X; $(X + 29)\ 4.1 = X \times 140$ and $X = 0.88$ mg *i.e.* 8.8% of the injected dicumarol. The total activity in the liver before extraction was 120 registers per min representing 8.6% of the activity injected. The close agreement between these two figures is direct evidence that the activity found in the liver, following the injection of radioactive dicumarol is essentially all in the form of unchanged dicumarol. This means that for the liver, the activity gives us a reasonably accurate measure of the dicumarol present.

In a further test, dicumarol isolated from liver (6.1 mg, specific activity 4.1 reg/min/mg) was converted to the diacetate (M.P. 250-252°C, mixed M.P. 250-252°C). Specific activity of the diacetate was 3.3 (found) and 3.3 (calc) regs min/mg.

Dicumarol and vitamin K. It has been shown by a number of workers(1), that simultaneous administration of dicumarol and vitamin K reduces the action of dicumarol. A series of mice were therefore injected with 0.25 mg dicumarol together with 1 mg of vitamin K (2-methyl-1, 4-naphthoquinone phosphoric acid ester, Synkavite). It was found that excepting for the liver, there was no significant difference in the activity found in tissues from these animals, compared to mice receiving radioactive dicumarol without vitamin K at the same time. The activity found in the liver is shown in Fig. 3. It can be seen that the activity twenty minutes and one hour after the injection was the same for the mice which received vitamin K as for those which did not. However, after the first

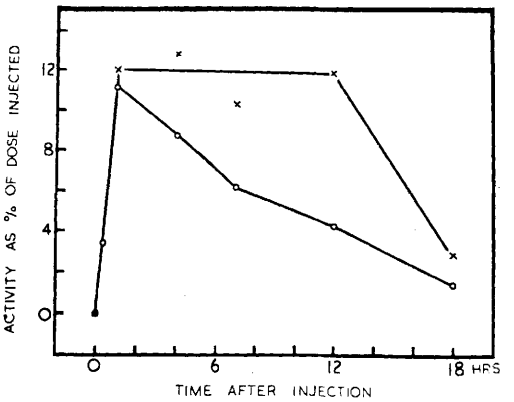

FIG. 3.

Dicumarol in mouse liver. x———x after intravenous injection of 0.25 mg C^{14}-dicumarol; o———o after 0.25 mg of C^{14}-dicumarol + 1.0 mg of vitamin K (2-methyl-1,4-naphthoquinone phosphoric acid ester).

hour the animals receiving vitamin K showed much less activity in the liver suggesting a more rapid displacement of dicumarol from the liver in the presence of vitamin K. Some dicumarol remained in the liver after 18 hours, at which time the values were about the same as for the animals without vitamin K.

Discussion. It is evident from these results that on administration, dicumarol was carried to the liver and remained fixed there for a considerable period. This period varied with the animal species and presumably this may be the cause of variations in the effectiveness of prothrombopenic agents between different animal species. Thus in the mice (Fig. 1) the prothrombin time reached its peak value on the third day and returned to normal values on the fourth day. In the rabbits (Fig. 2), the prothrombin time reached its peak value (38-42") on the third day but this value was maintained for 2 days and the prothrombin time did not reach normal values until the ninth day. It is significant that dicumarol remained in the mouse liver only 16 hours, and none could be detected after 24 hours, while the level of dicumarol in the rabbit liver remained constant (about 1 mg) for 3 days after the injection and a small amount was still present on the fifth and seventh days. This indicates a direct relationship between the duration of

fixation of dicumarol in the liver and the duration of the prothrombinopenia in the blood. Vitamin K appeared to interfere with the fixation of dicumarol in the liver in such a way that the dicumarol was displaced from the organ more quickly.

Since no radioactivity appeared in the urine for some hours after intravenous injection of labelled dicumarol, it is evident that in mice and rabbits, the dicumarol was not excreted by the kidney. This has also been reported by Weiner, Axelrod, Shapiro and Brodie(3) in man. However, the activity appeared in the bile and gastro-intestinal tract shortly after administration and much later it was found in the urine, indicating metabolism of the dicumarol. In the rabbit, all of the labelled groups in the dicumarol was reabsorbed and excreted in the urine but in the mouse, a part of it appeared in the feces. The nature of the product excreted is now under investigation. Thus far, it has been shown that the activity in the urine is not dicumarol, agreeing with the suggestion above that the dicumarol has been metabolised. In order to ensure complete absorption, the dicumarol used was administered intravenously. However, experiments have been conducted with the material given orally with essentially the same results.

Summary. Dicumarol, containing C^{14} in the methylene bridge was synthesized and given intravenously to mice and rabbits. The activity disappeared rapidly from the blood and was recovered in the liver, bile, intestinal contents and later in the urine, but not from other tissues. About 10% of the activity became fixed in the liver. It was demonstrated by isolation using the isotope dilution technique that this activity was due to unchanged dicumarol. This dicumarol remained fixed in the mouse liver for 16 hours, in rabbit liver for 3 days. The corresponding duration of hypoprothrombinemia was 4 days and 9 days. The presence of extra vitamin K resulted in the dicumarol disappearing more rapidly from the liver. It is suggested that the period of time that dicumarol remains in the liver is related to the effectiveness of the agent in interfering with the formation of prothrombin. Dicumarol was not excreted by the kidney but evidence of excretion of metabolic products was found in the urine and feces for the mouse and in the urine only for the rabbit.

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Relation of Adrenal Cortical Steroids to Antibody Release. (17839)

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That the lymphocyte is concerned with the manufacture and storage of antibodies has been demonstrated by a number of investigators(1,2,3,4,5). The administration to normal animals of those adrenal cortical steroids oxygenated at C_{11} or adrenotrophic hormone produces involution of the thymus, degeneration of lymphocytes in lymphoid tissue, a peripheral leucopenia accompanied by a granulocytosis and a decrease of lymphocytes in thoracic duct lymph(6,7,8,9,10,11,12,13). The lymphocyte has been shown to contain

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