only. We should mention that on microscopic examination two more animals in the testosterone-treated group and one more in the cortisone-treated group showed pulmonary micro-metastasis, which, had the animals been allowed to survive longer, would have grown to grossly visible size.

It is worth noticing that the local growth of the tumor was not a necessary condition for the appearance of the metastasis. This apparently paradoxical effect, first pointed out by AGOSIN et al.[2], was particularly evident in our estrogen-treated rats. Among them, the three animals with kidney metastasis did not show any sign of tumor growth in the tail and if we take the three groups together a significant incidence of mediastinal metastasis could be demonstrated in the absence of primitive growth. The microscopic picture of the metastatic growth did not show any peculiarity. In the lungs the tumor nodules were frequently centred by a vessel where a tumor emboli could occasionally be depicted. The growth in the kidneys and heart led to destruction of the organ structures and also infiltrates interstitially to a great extent.

The total absence of metastatic growth in all the twelve controls, despite the local growth, seems to exclude the view that the modification of the behaviour of this tumor was attained by its growing in the tail. Besides, the appearance of metastasis in treated animals which did not show local growth indicates that it is not only the easiness to penetrate voluminous vessels, in this case the lateral vein of the tail, which explains the modified behaviour[7]. Rather it seems that the hormonal treatment created certain conditions in the host that facilitated the arrest and proliferation of circulating tumor cells in sites which are usually unaffected in non-treated rats. The incidence of extra-pulmonary metastasis was highly significant in our material, showing that the pulmonary microcirculation is promptly traversed by the Yoshida neoplastic cells.

Direct experimental observation *in vivo* has shown that cortisone increases endothelial sticking of blood-borne tumor cells[8]. Cortisone also alters the lipid metabolism and increases blood coagulability[9]. As a matter of fact, the majority of the experimental factors that increase hematogenic metastasis produce a potential hypercoagulability and/or increases leucocytic sticking as pointed out by WOOD et al. and several others investigators[3,10–15].

The state of the endothelial surface and blood coagulability seem then to play a decisive role in the determinism of metastatic localization and growth of blood-borne neoplastic cells and are probably the preeminent host factors involved in the mechanism of metastasis formation[3,16]. We have so far no explanation regarding mechanism of action for the production of metastasis by treatment with sex hormones. However, it is not out of question to postulate that these hormones exert their action through some modifying effect on endothelial adherence and blood coagulability, as demonstrated for cortisone.

If this observation with the Yoshida sarcoma proves to be a general fact, a warning should be sounded concerning the utilization of sex hormones in clinical management of cancer patients, owing to a possible acceleration of metastatic dissemination in hormone-treated individuals, as has already been indicated in some recent papers[17–20].

Zusammenfassung. Es wird über die Verbreitung des metastatischen Yoshida-Sarkoms bei Ratten berichtet, die das Sarkom in den Schwanz geimpft erhielten. Die geimpften Tiere wurden mit Testosteron, Cortison und Östrogen behandelt. Die einfache Einpflanzung des Sarkoms ist nicht genügend, um die Verbreitung der Geschwulst zu veranlassen.

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Analysis of the Radioisotope Renogram[1]

The estimation of organ function, by external counting of γ-emissions from an administered radioactive substance, has been widely employed in thyroid studies. Since the kidneys accumulate and excrete certain radiolabeled dyes, the same type of approach is possible. The procedure which was evolved by WINTER[2] was termed the radioisotope renogram. Attempts at quantitation have been empirical thus far[3,4]. We wish to point out possible quantitation of the radioisotope renogram, based on a simple 4 compartment system.

Shown in the Figure is a possible model of the events following intravenous injection of a small quantity of a radioactive substance which is excreted by the kidneys (for example, the commonly employed 0.2 mg of *o*-iodohippuric acid represents only 7×10^{-7} moles; if distributed evenly in 5 l of blood, the concentration would be 1.4×10^{-7} *M*). At low concentrations there need be little concern with saturation of the renal excretory mechanism. Passage of the substance from blood to kidney and from kidney to urine, even if 'active transport', can be considered as a linear function of concentration at such great dilutions. It is also apparent that the reverse reactions (passage of material from kidney to blood, and from urine

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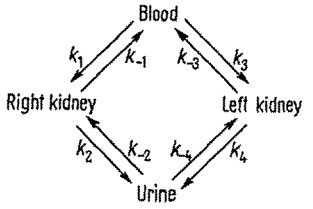
to blood) are negligible in the normal renal apparatus. Hence the rate constants k_{-1}, k_{-2}, k_{-3}, k_{-4} can be neglected, and we have a simple system: blood → kidneys → urine.



Simple model of the fate of a radioactive material injected into the blood stream as a bolus and excreted by the kidneys. The k's are the rate constants of the reactions.

The problem further simplifies if the two kidneys have identical function, or if one kidney is absent. In both of these simplifications the 4 compartments illustrated reduce to a 3 compartment model. The equations for solving such a system have been presented by HEINZ[5] and utilized in other studies[6]. In previous investigations the equations were used to predict blood levels of a material after depot injection. Here we will use the equations to calculate uptake at a depot (the kidney) after intravenous injection. The quantity of radioactivity in the kidney at any instant of time is:

$$q_t = q_i \frac{k_1}{k_1 - k_2} (e^{-k_2 t} - e^{-k_1 t}) \quad (1)$$

where q_t is the quantity at time t, q_i is the quantity injected, k_1 is the rate constant for transfer from blood to kidney, and k_2 is the rate constant for transfer from kidney to urine. The constant k_1 is estimated by taking the tangent of the renal curve at the first point of upswing. For example, the tangent at the point of upswing of the renal curve in a typical instance might be 60 U in 1.5 min, or 40 U/min. The theoretical maximal rise of the curves of both kidneys if there were no excretion, would be given by the standard set at the same geometry as the kidneys. In this case it amounts to 120 U. Hence the value of k_1 is 40/120 or 0.33/min.

Once k_1 is estimated, the time of attaining maximum height of the renal curve is read from the radioisotope renogram. Then k_2 is determined from:

$$t_{max} = \frac{1}{k_1 - k_2} \ln \frac{k_1}{k_2} \quad (2)$$

where t_{max} is the time of reaching maximum height, and ln is the natural logarithm. With k_1 and k_2 now determined, substitution can be made in equation (1) to calculate the expected height of the curve at any instant. Initial work has shown that k_1 has values of approximately 0.3 to 0.5/min, while k_2 is about 0.07 to 0.2/min. Two typical cases are listed in the Table with the observed and calculated heights of the curve at 3 points of time.

Reasons for deviation form such a simple model immediately came to mind.

(1) Even a well positioned renal probe will pick up radiation from the contralateral kidney. In addition, there is an anatomic asymmetry between the vasculature, and this is seen by the two probes[4]. Non-renal vasculature contributes to the curves, and even nephrectomized individuals will show a slight curve as the radioactive bolus passes by.

Data on the excretion of *o*-iodohippuric acid-I^{131} by 2 individuals as recorded by the radioisotope renogram. Predicted values were calculated from equation[2].

	T_{max} min	Time min	Chart deflection units observed	predicted
J.G. 29, female	4.1	4.1	52	52
Right and left identical kidney traces		10.0	31	31
		20.0	16	7
T.T. 66, male	5.0	5.0	79	79
Right kidney absent		10.0	58	61
		20.0	47	42

(2) All of the material (typically *o*-iodohippuric acid) is not excreted according to such a simple formulation. A small fraction is blood or renal bound[4] and has a longer residence in the body. This will cause deviation toward a less rapid fall of the renal curve at longer times. Such a prediction has been borne out by several observations (and see the last figures in each case in the Table). It may be, however, that deviations from the expected simple curve will be of value in providing insight as to blood or renal binding of the radioactive material under use.

(3) The calculation of k_1 depends upon accurately knowing the height of the standard when viewed by the renal probes. The 2 kidneys may vary in both depth and shielding by the vertebra and perirenal fat. Estimation of the standard at such a depth and shielding may be difficult. One can use the empirical approach that the 'corrected' standard is that value which allows the calculated value of k_1, when coupled with t_{max}, to determine the value of k_2 to predict the shape of the curve.

(4) The rate constant k_2 represents not only transfer, but also flushing of the radioactive material from the kidney tubules by the urine flow. Hence k_2 should be partially dependent upon the volume of urine flow (it is a composite constant). That this is reasonable is shown by the fact that dehydrated individuals have been noted to have a prolonged excretory phase.

(5) The calculations have been based on first order assumptions, with neglect of the reverse flow constants (k_{-1}, k_{-2}, k_{-3}, k_{-4}). While this assumption is valid for the normal kidney, it does not necessarily hold for diseased kidneys. In such instances, reverse flow may occur, and the full 4 compartment (8 constant) system must be evaluated.

Zusammenfassung. Beim Fehlen einer Niere, und bei zwei normalen Nieren von gleicher Aktivität sind die Radioisotopen-Renogramme (Modell mit drei Bestandteilen: Blut, Niere und Urin) genau beschrieben worden.

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