a time for 2 hours in a bell jar of 3.5 liters capacity through which was flowed from bottom upwards a mixture of dry nitrogen and air containing 6% oxygen. A dental anesthesia machine was used to mix the gases and provide constancy of flow which was at a rate estimated at more than 1 liter per minute. A Hay's gas analyzer having graduated measurements of 0.2% was used to measure oxygen and carbon dioxide percentages at inlet and exhaust orifices of the bell jar. Carbon dioxide concentration never reached a measurable level and oxygen concentration remained at six percent; humidity, however, was not standardized in this apparatus. A nitrogen-oxygen mixture at sea level (atmospheric pressure, 760 mm mercury) containing 6% oxygen gives approximately the same concentration of oxygen molecules as exists in the atmosphere at 30,000 feet above sea level (atmospheric pressure, 226 mm mercury). As with mice exposed to low atmospheric pressure(2), test mice upon return to room air showed no immediate ill effects from the exposure and appeared well throughout pregnancy.

The 10 remaining mice were kept as controls under usual laboratory conditions. Fetuses were removed from mothers by section during the 19th day of gestation, and skeletal systems were stained with alizarin red for examination after clearing of soft tissues with potassium hydroxide.

Results. Exposure of pregnant female mice on 10th day of pregnancy for 2 hours to a 6% oxygen-94% nitrogen mixture at normal atmospheric pressure (760 mm of mercury) resulted in malformations of the kind found after exposure to a low atmospheric pressure—226 mm of mercury, which in respect to number of oxygen molecules per unit volume of air is the equivalent of 6% oxygen in nitrogen at sea level. Hemivertebrae, ablations and fusions of ribs and vertebrae (Fig. 1) were found in 26% (15 of 57 young) of the progeny of 12 females. None of 52 young from control animals kept in ordinary atmosphere at sea level showed similar defects.

Summary. Pregnant mice kept for 2 hours during the 10th day of gestation in a gas chamber containing 6% oxygen in nitrogen at normal atmospheric pressure produced progeny having malformed ribs and vertebrae.

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Transcapillary Exchange of S^{35}-Labeled l-Methionine in the Human Forearm.* (22863)

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Previous reports from this laboratory have been concerned with the transcapillary exchange of water and electrolytes in different vascular areas of the body(1,2). A technic was described utilizing the forearm vascular bed where the transcapillary exchange of labeled substances could be studied in some detail. Observations could be extended over a

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TABLE I.

Patient	Age	Diagnosis	% loss around peak of dye curve		% loss of methionine	
			SCN	Methionine	30 sec. past peak	At equilibrium time of SCN
C.C.	32	Peptic ulcer	45	51	55	44
B.N.	50	Conv. pneumonia	30	11	33	40
G.K.	28	Psychoneurosis	47	41	54	45
G.S.	35	Irr. colon	44	36	39	34
W.A.	29	Pyelonephritis	49	11	42	28
P.N.	44	Peptic ulcer	41	14	49	49
B.B.	26	Psychoneurosis	40	18	49	24
E.B.	57	Gastroenteritis		56	49	
J.D.	71	Bronch. ca.		28	52	
J.W.	48	Essen. hypert.		19	54	
		Mean and S.D.	42.3 ± 6.3	28.5 ± 16.7	47.6 ± 7.3	37.7 ± 9.3

relatively long period of time since contamination by significant recirculation of the labeled materials could be avoided(3). It was found that water and thiocyanate ion crossed the capillary membrane from blood to tissue at net rates characteristic for each substance and that return of these substances from tissues to blood occurred relatively rapidly(3).

It seemed of interest to extend these studies to include an organic substance which is utilized in cellular metabolism in order to determine whether the pattern of transcapillary exchange of such a substance might differ from that observed previously with thiocyanate ion and isotopic water.

Methods. The present studies were performed on 10 male patients with various disorders as listed in Table I. Their average age was 42 with a range from 26 to 71 years. The method of administration and collection of samples, which has been described previously (1), consists of injecting into the brachial artery a mixture of the substance under study, followed by continuous sampling at intervals of 2 to 4 seconds from an ipsilateral antecubital vein. The dye T-1824, and sodium thiocyanate were given in the same amounts as described previously(1). To this mixture S^{35}-labeled l-methionine was added to provide an injected activity of 25 to 30 μc. Periodic samples from a contralateral vein revealed that the concentration of recirculating tracer materials was insignificant. Analytical procedures for determining concentrations of T-1824 and thiocyanate have been described(1). Aliquots of plasma samples were diluted with equal parts of isotonic saline solution and 0.5 ml of each was then pipetted into aluminum planchets in order to count S^{35}-beta emissions. Aliquots of the injected mixture first were diluted 200 times with isotonic saline solution, mixed, and an aliquot of this was diluted with equal parts of plasma containing no radioactivity. 0.5 ml of the latter was also transferred to a planchet. Samples were dried at room temperature and counted, using a thin, end-window ("Micromil") gas flow counter.‡ The method for calculating the net transcapillary losses of thiocyanate has been described(1,3). The same method was applied to S^{35}-labeled l-methionine. Correction for red cell penetration of methionine was determined *in vitro* by comparing the activity of S^{35}-labeled l-methionine added to red cell free plasma with that added to whole blood. The amount added was .002 μc/ml plasma or whole blood. Whole blood samples were allowed to equilibrate one hour, then centrifuged, and the activity of the supernatant plasma and also of the reference plasma samples determined as described above. Counts/min/ml in supernatant plasma multiplied by the plasmatocrit gave total counts in the plasma fraction of 1 ml whole blood (C_{pwb}). Red cell penetration (C_{rc}) was the difference between C_{pwb} and the counts/min in 1 ml of reference (red

‡ Model No. D-47, Nuclear Instrument Corp., Chicago, Ill.

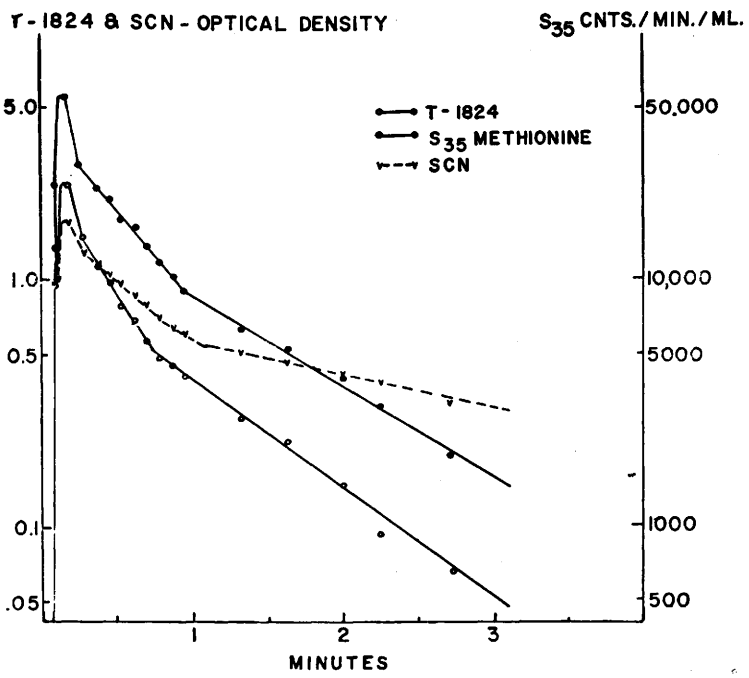

FIG. 1. Time concentration curves obtained from antecubital vein after inj. of a mixture of the dye T-1824, sodium thiocyanate and S^{35}-labeled l-methionine into the ipsilateral brachial artery in subject G.S. The T-1824 and methionine downslopes are nearly parallel. The thiocyanate curve deviates from the others soon after the peak. See text for further details.

cell free) plasma. The mean value for C_{rc}/C_{pwb} in blood samples drawn from 5 individuals was 0.54 (range .47 to .64). The penetration factor was expressed as the fraction of red cell water available for equilibration with plasma water and was obtained by multiplying the mean value by the ratio of the water content of plasma to the water content of red cells (0.54 x .90/.65 = .75). This penetration factor (0.75) was multiplied by $\frac{cWc}{pWp}$ [see equation 4 of the original description of methods(1)]. In the case of SCN a penetration factor of 0.70 rather than 1.0 was used in calculating the expected concentration of the thiocyanate(2).

Results. I. *Characteristics of time-concentration curves.* The downslopes of time concentration curves of S^{35}-labeled l-methionine differed from those previously observed with deuterium oxide and thiocyanate ion(3). The methionine downslope paralleled the T-1824 downslope much more closely than thiocyanate ion (Fig. 1). This parallelism usually persisted beyond equilibrium time (3) of thiocyanate and only when the concentration of injected substances had reached almost trace levels did the methionine curve assume a distinctly shallower slope than the T-1824 curve. In 7 instances the early portion of the methionine downslope was somewhat steeper than that of T-1824.

Plotting "expected" and actual concentrations(3) of labeled methionine revealed a delayed equilibrium time and only insignificant return as compared to thiocyanate (Fig. 2). In contrast to SCN and D_2O, a half return time(3) could not be calculated for methionine because of the late onset and small quantity of return during the period of observation.

II. *Transcapillary Loss.* In the case of thiocyanate and deuterium oxide, the greatest transcapillary loss occurred near the peak of the concentration curves at a time when the concentrations in blood exceeded that in extravascular spaces(3). In the case of labeled methionine the per cent loss at the peak was somewhat variable and usually rose to a higher level during the half minute follow-

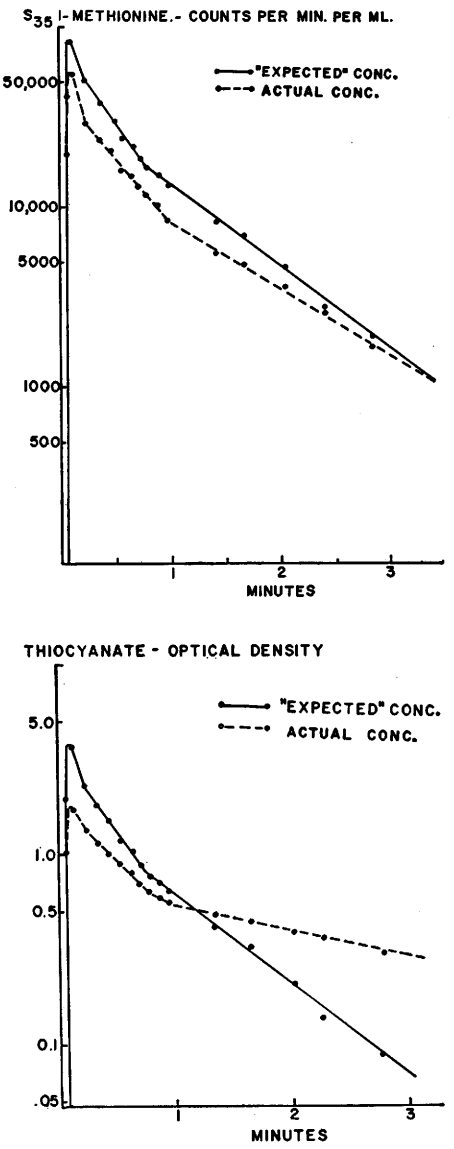

FIG. 2. "Expected" and actual concentration curves of S^{35}-labeled l-methionine (above) and of thiocyanate (below) derived from Fig. 1. See text and reference 3 for further details. This case did not show the increasing % loss in the portion immediately following the peak as did most of the other cases.

ing the peak where it remained fairly constant for a considerable period. Thus, the mean per cent transcapillary loss of labeled methionine at the peak in the 10 cases studied was 28.5 with a large standard deviation of 16.7. The per cent loss at a point 30 seconds beyond the peak concentrations averaged 47.6 ± 7.3. The small percentage return of these transcapillary losses is indicated in the 6 cases in which SCN was determined simultaneously. At the equilibrium time of thiocyanate [point at which net return equals net loss(3)] the net exchange of labeled methionine averaged 37.7 ± 9.3% loss from blood to extravascular spaces.

The per cent loss of thiocyanate ion observed in these studies was in the same range as that previously reported(3). In the 6 cases studied in the present experiments the mean transcapillary loss at the peak was 42.3 ± 6.3%.

Discussion. The observed transcapillary losses of labeled methionine indicated that this substance diffused relatively freely through the capillary walls. Despite this free diffusion, however, the net return from extravascular space to intravascular space was minimal during the period of observation. These facts suggest that the labeled methionine became bound after leaving the circulation in such a way as to prevent its return. This would explain the persistence of maximal rates of net transcapillary loss despite a constantly diminishing concentration in the blood. The most reasonable site of such binding would be metabolic incorporation into cells. Cowie and Roberts have presented evidence for a high degree of metabolic binding of S^{35}-labeled amino acids in bacterial cells (4). Intracellular penetration and binding of l-methionine would explain the difference in the pattern of transcapillary net exchange as compared to the largely extracellular thiocyanate ion. It is significant that other small molecules which have primarily an extracellular distribution, such as S^{35}-labeled sulfate and radiosodium exhibit transcapillary exchange patterns resembling thiocyanate ion (5).

Still a third pattern of transcapillary net exchange was exhibited by heavy water which showed early transcapillary losses averaging 90%, a delayed peak compared to T-1824 and equilibrium and half-return times intermediate between SCN and methionine(3). These differences between the diffusion patterns of D_2O and methionine can be ac-

counted for on the basis of the following characteristics of D_2O: (1) extracellular as well as intracellular distribution, (2) unusually high diffusibility through capillary walls, and (3) escape of a considerable portion from metabolic binding.

The mechanism for the frequently observed increasing per cent net transport during the early period of the downslope of l-methionine is not apparent. Possible explanations might be that metabolic incorporation is not as efficient when peak concentrations are present, or that the later downslope represents plasma moving at lesser velocity permitting more complete transcapillary exchange.

Summary and conclusions. 1) In the forearm the maximum transcapillary loss of S^{35}-labeled l-methionine averaged 47.6 ± 7.3%. Methionine exhibited a pattern of transcapillary net exchange which is consistent with intracellular binding. A high rate of net loss was observed for a much longer period than with thiocyanate ion and the equilibrium time was delayed considerably. Net return to the circulation was small compared to thiocyanate during the period of observation. 2) On the basis of these and other observations it is suggested that 3 distinct classes of substances have characteristic patterns of transcapillary net exchange which may be distinguished with the present technic. These substances are (a) primarily extracellular ions, (b) primarily intracellular diffusible substances, and (c) highly diffusible molecules which are distributed throughout both compartments (body water).

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Changes in Plasma Prothrombin, Ac-Globulin, and Antithrombin Concentration Following Intravenous Administration of Estrogens.* (22864)

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Recent observations made in clinics indicate that estrogens given intravenously are of therapeutic value for control of spontaneous hemorrhage, such as epistaxis, excessive postoperative bleeding from tonsillectomies and, on occasion, from urological surgical procedures(1,2). Moderate success has also been reported for this therapy in spontaneous gastrointestinal bleeding. These uses are of more recent application than for control of uterine bleeding for which estrogens have had long popularity. The studies here reported are regarded as a preliminary survey designed to determine what changes may be produced in concentrations of blood coagulation factors following intravenous injection of estrogens. Previous investigators reported alterations of clotting factors coincident with physiological changes in levels of estrogenic hormones, as well as a rise in heparin like antithrombinic activity with administration of synthetic estrogen(3). For a brief period ovarian extracts were given extensive trials for control of bleeding tendency in hemophilia(4), but this did not become established practice(5). During pregnancy there is elevation of prothrombin level(13) near term as well as in

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