experiments in which the faster migrating group of human γ_{2a}-globulin molecules (Fig. 1) fail to sensitize heterologous skin. Further investigation will be required to determine if the human, mouse and guinea pig subclasses are comparable.

Summary. The γ_{2a}, γ_{2b}, and γ_{2c}-globulin subclasses of human serum IgG were tested for their ability to sensitize heterologous (guinea pig) skin. In studies using 15 G-myeloma proteins, positive RPCA tests were obtained with γ_{2b} and γ_{2c}-myeloma proteins. In contrast, γ_{2a}-myeloma proteins gave negative RPCA reactions. These results indicate that the heavy polypeptide chains of γ_{2a}-globulin molecules differ from the heavy chains of γ_{2b} and γ_{2c}-globulin molecules in skin sensitizing as well as in antigenic properties.

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Relationship Between Lymphatic and Blood Flow in Various Structures in the Abdominal Cavity.* (29733)

RITA L. PALDINO AND CHESTER HYMAN
Department of Physiology, University of Southern California, School of Medicine, Los Angeles

It is generally taught that lymph flows from the tissues of the lower extremities and the abdominal organs into the cisterna chyli and then through the thoracic duct to empty into the venous system at the root of the neck. Based on this concept the fraction of various substances absorbed from the alimentary canal into the lymphatic system has been calculated from concentrations and flows in the thoracic duct(1,2,3). The persuasive morphological evidence(4,5) that lymph might empty into the venous system by alternative lymphatico-venous anastomoses has been disregarded(6). We have adduced pertinent information bearing on this point from studies of tissue clearance of diffusible and non-diffusible molecules.

Hyman and Freedman(7) found that the rates of removal of a small dye molecule (Water Blue) and a protein-bound dye (T-1824 albumin) from microinjection sites in frog mesentery differed, but the ratio of clearance of diffusible to clearance of colloidal

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compounds was not nearly as great as corresponding ratios from other tissues (*e.g.* 8). If small molecules are cleared primarily by transfer across the capillary wall while colloidal molecules are removed exclusively by the lymphatics, these data imply a lymph flow about 10% of the effective blood flow in this tissue. In view of the rapid lymph flow in the entire frog(9) this fraction, although high, might be explained by the somewhat higher pressure in the capillaries of the mesentery. Independent measures of effective blood flow and lymph flow in mesentery are needed to establish the basic assumptions and implications of the data.

The rates of disappearance of diffusible and colloidal dyes from micro-injection sites were measured in the rat mesoappendix; lymph flow rates were checked by direct cannulations of the "iliocolic" and thoracic ducts of identical series of animals; and finally, thoracic duct lymph flow was measured during alternating periods of occlusion and release of the "iliocolic" duct. The results of all these experiments are consistent with the hypothesis that much of the lymph from the abdominal viscera returns to the circulation at some point(6) distal to the entry of the thoracic duct. Our findings argue in favor of lymphatico-venous anastomoses throughout the abdominal venous system. Under the conditions of our experiment these "taps" apparently play a major role.

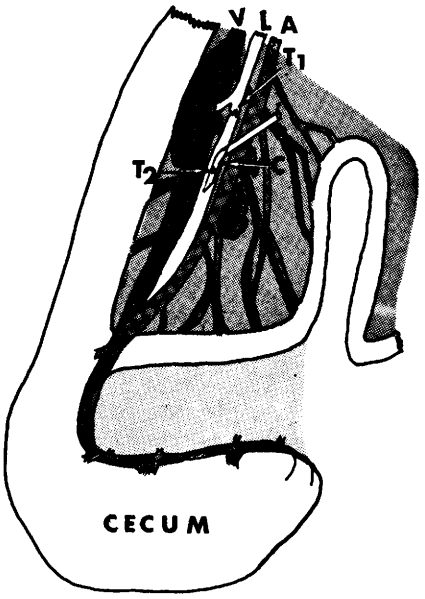
Methods. Two experimental procedures were used: A) the micro-tissue clearance technique for measurement of disappearance rates of colloidal and diffusible dyes from rat mesoappendix and B) direct measurements of lymph flows from cannulated "iliocolic" and thoracic ducts.

A) The clearance technique was modified from that described in an earlier paper(10) for application to the rat spino-trapezius muscle. Zweifach's rat mesoappendix preparation(11) was used with added provision for micro-injection into the lower surface of the mesentery and for irrigation of the exposed tissue by a drip whose temperature could be varied and thermostatically controlled. For each determination a single micro-injection (0.05-0.07 μl) of 8% Water Blue or dye-labelled protein was made directly into the mesentery. The dye-labelled protein was prepared by mixing Evans Blue (Warner-Chilcott) with bovine serum albumin to give a solution containing 4 mg of dye and 25 mg of protein per ml; no more than 10 moles of dye per mole of albumin were present so that the system contained no free dye.† Since temperature is one of the factors known to modify both lymph and capillary flow, this variable was used in pilot studies. Clearance of both markers were determined at drip temperatures ranging from 29 to 43°C.

The influence of intentional modification of capillary and lymph flow on these clearances was determined in the next series of experiments. After measuring the control clearance rate of Water Blue at a drip temperature of 33°C, the blood perfusing the iliocolic angle was diluted by infusion of either physiological saline (0.9%) or 12% bovine serum albumin in 0.9% NaCl into a distal mesenteric artery at a rate of 0.5 ml/min for 20-30 minutes. Repeated measurements of the clearance rate were made several times during the infusion and at intervals after its termination. In a second series of experiments T-1824 labelled albumin was used as the marker and the drip temperature was elevated to 37°C. These respective temperatures were chosen because (Table I) clearance of Water Blue at 33°C approximates clearance of the protein at 37°C.

B) The lymph flow rates were studied using 80-100 g rats anesthetized with 3.0-3.5 mg sodium pentobarbital given intramuscularly. The "iliocolic" ducts were cannulated in one group of animals by a modification of the technique of Baez(13). The caecum and about 3 cm each of the ascending colon and ileum were exteriorized through a small midline incision and spread over saline moistened gauze. The lymph duct becomes readily visible when the largest mesenteric node is dissected free from adherent mesentery. With the aid of dissecting glasses, the lymph vessel is isolated from the accompanying ar-

† This was established by dialysis experiments on occasional batches and agrees with the studies of Rawson(12).



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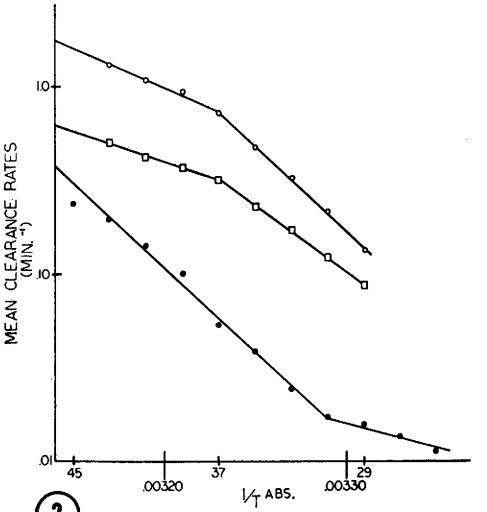

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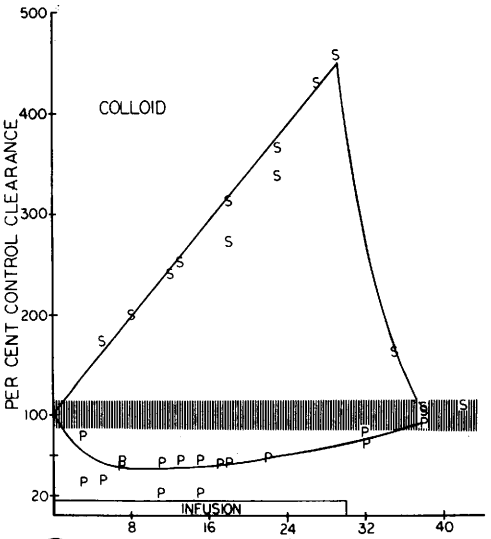

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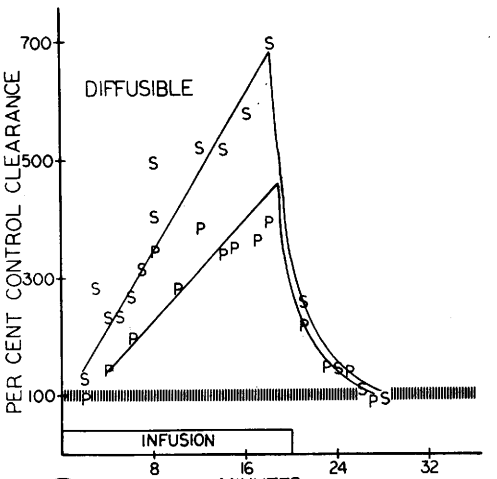

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FIG. 1. Schematic drawing of the rat mesoappendix preparation with lymph duct cannulated. V, L, and A = iliocolic vein, lymph duct, and artery respectively; T_1 and T_2 = first and second ligatures; C = cannulus.

FIG. 2. Arrhenius plot of mean clearance rates of Water Blue from mesoappendix (open circles), T-1824 albumin (open squares) from rat mesoappendix, and Water Blue (closed circles) from rat spino-trapezius muscle. Breaking points in the relationships occur at approximately normal temperature of the particular tissues, 31°C for muscle and 37°C for mesentery. Note that curve for muscle shows a higher temperature coefficient (slope) to the left of breaking point, while the curves for mesentery have their higher slopes to the right of break.

FIG. 3. Percent change in T-1824 albumin clearance from rat mesoappendix during infusion

TABLE I. Mean Tissue Clearance Rates Measured at Various Temperatures.

Temp (°C)	K_{alb} (min^{-1})*	K_{wb} (min^{-1})†	K_{wb}/K_{alb}	K_{wbm} (min^{-1})‡
29	.080 ± .006§ (5)	.133 ± .037§ (8)	1.66	.0126
31	.123 ± .027 (5)	.214 ± .016 (5)	1.74	.0140
33	.172 ± .024 (6)	.324 ± .100 (14)	1.89	.0199
35	.229 ± .015 (6)	.482 ± .150 (11)	2.10	.0319
37	.322 ± .025 (5)	.728 ± .150 (13)	2.26	.0533
39	.369 ± .056 (5)	.930 ± .090 (9)	2.52	.1225
41	.423 ± .042 (5)	1.080 ± .038 (8)	2.56	.1719
43	.505 ± .012 (5)	1.301 ± .210 (10)	2.57	.2358

Figures in parentheses are number of individual experiments.
* K_{alb} = clearance of labelled albumin from mesoappendix.
† K_{wb} = clearance of Water Blue from mesoappendix.
‡ K_{wbm} = clearance of Water Blue from muscle.
§ Standard deviation.

tery and vein and a ligature tied about the duct just below the tributary that connects it to the large node (Fig. 1). A second ligature is placed about 5 mm caudad to the first and the beveled end of a polyethylene tube (P.E. 10) is passed into the duct for about 4 mm through a small longitudinal opening between the ligatures and then tied securely in place. The organs are replaced in the abdomen and the incision closed with wound clips.

In another series of animals the thoracic duct was cannulated by Bollman's technique (14). The P.E. 50 polyethylene cannula was tied firmly in place in the vessel and then positioned to lay straight in the duct, but to arch along the diaphragm and pass out of the abdominal cavity laterally just posterior to the last rib. Immediately thereafter the "iliocolic" duct was isolated and a loose ligature was placed about it. The iliocolic angle of the intestinal tract remained exposed in saline moistened gauze and maintained at body temperature throughout the experiment. Thoracic flow was measured continuously while the "iliocolic" duct was occluded at intervals by applying tension on the ligature. Before and after each occlusion period the patency of the smaller vessel was checked.

The experiments, which lasted from 1.5 to 3.0 hours, were begun after a 30-minute stabilization period. Five to 10 minute lymph samples were collected in weighed plastic tubes, and flow rates calculated in g/hr.

Results. The effects of local temperature on clearance rates of diffusible and non-diffusible molecules from micro-injection sites in rat mesoappendix are summarized in Table I. Previously published data on clearance of Water Blue from skeletal muscle(10) are included in the last column of the Table. At all temperatures clearance from the mesoappendix is considerably higher than that from muscle of the same animal and of the same magnitude as clearance from frog's mesentery (0.55 min^{-1}). These findings further support the earlier suggestion that effective blood flow in mesentery is much more rapid than in muscle(7). The relationship between temperature and clearance of diffusible and colloidal molecules is described in Fig. 2, an Arrhenius plot of the mean clearance values. In mesentery, the curves for both markers change slopes at 37°C; the temperature coefficient was 2.0×10^4 in the lower temperature range and 0.9×10^4 in the higher range. The relationship for Water Blue clearance in muscle breaks at 31°C and the shift in temperature coefficients is reversed, *i.e.* the higher value is at the higher temperatures and lower value at the lower temperatures.

At every temperature clearance of labelled albumin is more than 30% of the diffusible ion clearance rate. The ratios of diffusible to

of physiological saline (S) and 12% albumin solution (P); shaded area represents range of control clearances at 37°C.

FIG. 4. Percent change in Water Blue clearance from rat mesoappendix during infusion of physiological saline (S) and 12% albumin solution (P); shaded area represents range of control clearances at 33°C.

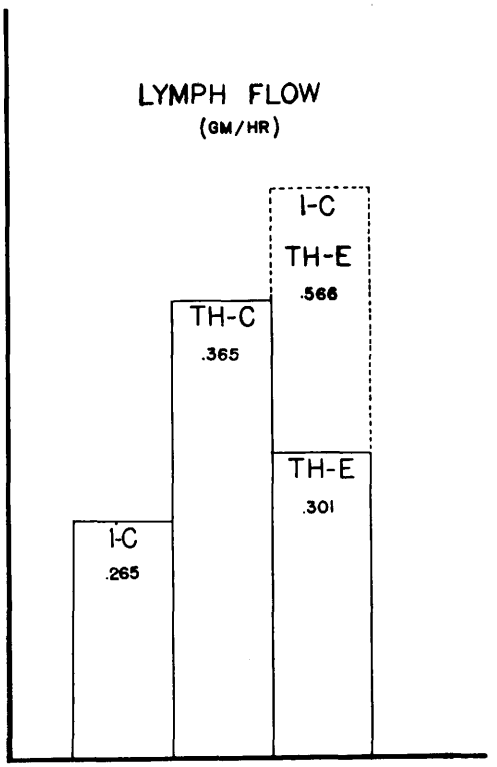

FIG. 5. Mean lymph flow rates measured from directly cannulated ducts. I-C = flow from "iliocolic" duct; TH-C = normal flow from the thoracic duct; TH-E = thoracic flow with occlusion of the "iliocolic" duct; I-C + TH-E = calculated normal thoracic flow if all of the "iliocolic" lymph were emptying into the cisterna chyli.

colloidal clearance rise in a linear fashion from 29 to about 39°C, but approach an asymptote at the higher temperatures.

A typical experiment comparing infusion of saline and a hyperoncotic solution on clearance of *dye-labelled protein* from rat mesentery is represented in Fig. 3. Note that clearance is decreased during the protein infusion but significantly increased during saline infusion. Fig. 4 shows that removal of the *diffusible molecule* is markedly augmented with infusion of either solution. In all cases the clearance rates return to pre-infusion levels within 10 minutes after the infusion is terminated. Qualitatively comparable results were obtained in experiments of this type on 8 animals.

The mean lymph flow rates as measured from directly cannulated ducts in rats are summarized in Fig. 5. Normal thoracic lymph flow rates in 30 cases was 0.365 g/hr and normal iliocolic lymph flow was 0.265 g/hr. When iliocolic flow was obstructed, thoracic flow fell to 0.301 g/hr, about an 18% drop from normal. Calculations show that only about ¼ of all the lymph coming from the iliocolic angle passes into the thoracic duct.

Discussion. A functional relationship between clearance of colloidal substances from local injection sites and lymph flow rate is supported by the experiments with infusion of saline and hyperoncotic solutions. Since there is no clear evidence that extra-vascular colloids can be restored to the circulation across the intact capillary wall, one must assume that the dye-labelled protein returns to the vascular system via the lymphatics. This is an extension of Drinker's dictum(15) that the chief "function of the lymphatics is apparently the unremitting removal from the tissues of excess blood proteins." If this assumption is valid, removal of such a marker is a reasonable index of lymph flow in the tissue under study. However, because movement of these large molecules is probably impeded by the structural elements of the mesenteric tissue, the exact quantitative relations remain uncertain.

Several workers suggest that protein may move from the extravascular space directly into the capillaries. Jepson *et al*(16) reach this conclusion because they found clearance of radio-iodine labelled albumin from subcutaneous tissue exceeded the largest estimate of local lymph flow. But Hollander(8) found that RISA is cleared from subcutaneous tissue at rates that *are* consistent with lymph flow. Jacobssen and Feldman(17) reported that labelled protein injected into knee joints of dogs appeared in the vascular system much before it could be detected in the thoracic duct; they concluded that the label must have entered the circulation by direct capillary transfer. These results can be explained if the protein from the joint passes through the local lymphatics and then is short circuited into the circulation through peripheral lymphatico - venous connections before it reaches the point of sampling in the thoracic duct. This alternative explanation is consistent with morphological data.

The results of the studies summarized in Fig. 3 clearly indicate a relationship between lymph flow and colloid clearance. With infusion of saline the increase in local capillary pressure and area, as well as the decrease in colloid osmotic pressure, would favor an augmented filtration and hence lymph flow rate. Under these circumstances there was always a significant increase in clearance of colloid. Infusion of the hyperoncotic solutions might give an equivalent increase in capillary pressure and area, but the elevated colloid osmotic pressure in the capillary would tend to diminish filtration and lower lymph flow rates. Under these circumstances clearance of colloid was always subnormal.

The significance of clearance of small diffusible molecules from tissues has been discussed (e.g. 18, 19, 20) and is, at least tacitly, accepted as a reasonable measure of effective blood flow. The more rapid clearance of Water Blue from frog mesentery(7) and rat mesoappendix than from rat muscle (10) probably reflects the high ratio of capillary blood flow to the volume of distribution of the label in the almost 2-dimensional mesentery. The extraordinarily high rate of lymph flow in the mesentery might be explained by: 1) dilution of plasma in local vessels by absorption of water from the gut, but this could not account for changes in the *mesoappendix*; 2) absorption of the irrigating fluid (a protein containing saline solution) from the surface of the mesentery; or 3) the characteristically high capillary pressure and capillary permeability could increase filtration. The decrease in clearance of colloidal markers with hyperoncotic infusion is evidence that modification of capillary filtration rates has considerable influence on lymph flow.

Filtration of such a large fraction of the circulating fluid should significantly increase the hematocrit of the venous blood draining the gastrointestinal tract. Preliminary comparisons of arterial and *portal* vein hematocrits showed only a slight increase, but more careful studies of *mesenteric* venous blood showed at least a 30% higher hematocrit than simultaneous arterial samples.

The production of about ⅔ of the total thoracic flow by this small, localized area of the intestinal tract and the failure of obstruction of this tributary to decrease flow significantly from the major trunk, suggest that much of the lymph formed in the region of the iliocolic angle never reaches the cannula in the thoracic duct. Lymphatico-inferior caval or portal "taps" in the abdominal region probably provide a path for restoring this lymph to the circulation.

Gross connections between lymphatics and veins, other than the classical thoracic and cervical ducts, were described in the human as early as 1898(21), and later in monkeys (22), in the rat(23,24), in cats(25), in newborn fetuses(26), and in dogs(27). In general, taps have been most commonly described in the region of the renal veins.

Freeman(28) concluded that the presence of "taps" insures adequate drainage of lymph from the intestines. Most recently Threefoot *et al*(5) demonstrated lymphatico-venous and lymphatico-lymphatic communications 21 to 32 days after ligation of the cisterna chyli, or much sooner in animals pretreated with hexamethonium. Rusznyak *et al*(29) conclude that such lymphatico-venous anastomoses exist, but normally have limited function; they believe that alternative lymphatico-venous "taps" are physiologically significant only under special conditions. Thus, the signficance of these connections varies from time to time and situation to situation. It is, therefore, essential that the existence and the consequences of a significant lymph flow via these alternative lymphatic venous "taps" be considered.

Conclusions. 1. Clearance of small solutes from the mesentery is about 10 times as rapid as clearance of similar materials from muscle. 2. The ratio of clearances of colloidal to diffusible molecules from mesentery is about 2:3 at 29° and decreases to 1:2.5 at 43°C. 3. Experimentally induced increases of capillary filtration, and presumably lymph formation, increase clearance of colloidal material; those diminishing capillary filtration decrease clearance of colloids. 4. Movement of large molecules from injection depots may serve as an index of local lymph flow; clearance of small diffusible molecules measures

flow through vessels capable of exchange. 5. Direct determinations of lymph flow rates from the "iliocolic" duct were found to be almost as great as flow from the thoracic duct. However, occlusion of the "iliocolic" duct diminishes thoracic duct flow by only a very small fraction. This paradox can be explained by the existence of connections between abdominal lymphatics and veins.

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Mycoplasma (PPLO) from Covertly Contaminated Tissue Cultures: Differences in Arginine Degradation Between Strains.* (29734)

PAUL M. KRAEMER† (Introduced by H. Koprowski)
Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

Mycoplasma isolated from covertly contaminated tissue cultures were divided into two types according to their effect on L5178Y cell cultures(1). The growth of "lytic" strains in such cultures was accomplished by a complete cytocidal response. Non-lytic strains also grew well, but this growth had no discernible effect on growth of the cells. Neither lytic nor non-lytic strains produced any evident cytopathology when inoculated into monolayer cultures of monkey kidney, HeLa, or early passage cultures of mouse, hamster, or chicken embryos. Thus, all of these strains differed from those reported by Stern *et al* which showed a pronounced cytopathic effect on various monolayer cultures (2).

The cytocidal response of L5178Y cell cultures to the "lytic" strains has been asso-

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† Present address: Biomedical Research Group, Los Alamos Scientific Laboratory, Los Alamos, N. Mex.