B_{12} preceded by one of these undiluted compounds evacuated from the stomach at a much slower rate than without pre-administration of the compound. Saline cathartics such as hypertonic solutions of magnesium sulfate and disodium phosphate also enhanced absorption of Vit. B_{12} when administered in the same way. However, when tested with "intestinal loop" technic, neither surfactants nor salt solutions were able to substitute for gastric intrinsic factor. The possible mechanisms for the observed phenomenon of enhancement of absorption have been discussed.

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Plasma Protein VII. Site of Degradation of Serum Albumin.* (25606)

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We reported(1) inability to find significant breakdown of homologous albumin labeled with C^{14} *in vitro* in any slice or homogenate system prepared from rat tissues, with the exception of spleen homogenate. These results are in accord with those obtained by Katz and Sellers(2), but disagree with those of Roberts and Kelley(3) who reported rapid breakdown of serum albumin in slices prepared from rat livers. However, it is necessary to postulate that breakdown occurs at some site in the animal to account for the rapid turnover of serum albumin observed in numerous investigations *in vivo*(4). It is possible that albumin and perhaps other plasma proteins continually 'leak' into the gastro-intestinal tract where they are hydrolyzed. Cellular barriers are permeable in many cases to plasma proteins. Thus, both albumins and globulins pass rapidly from plasma into lymph in all species which have been investigated(5,6,7); they pass from the circulation into the peritoneal cavity and back(8,9,10) and into milk (11,12). It has also been shown that, in the guinea pig, plasma proteins are transferred across the placenta to the fetus(13), and, as is well known from immunological investigations and recent isotopic work, globulins are absorbed from the gut of newly born animals (14). To demonstrate such a loss of albumin into the gut from the blood stream, 2 types of experimental method were used, (a) the washing method, and (b) the freezing method. In both methods rabbits were first injected with either albumin-I^{131} or iodide-I^{131}. In the washing method, the lumen of an isolated section of the gut was continuously washed for several hours with a dilute albumin solution; in the freezing method an isolated section of gut was injected with an albumin solution; the section was immediately excised and frozen in an acetone-dry ice bath. While still frozen the tissue was removed leaving the core of frozen albumin-containing solution. Distribution of radioactivity in the albumin solutions so obtained was investigated. It is assumed that any radioactive albumin passing from the circulating blood into the gut would be trapped in the large volume of unlabeled albumin solution, and in the short time before enzyme action was stopped with trichloro-

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acetic acid (TCA), or freezing it would not be extensively broken down.

Methods. The iodinated albumin (bovine or rabbit, 100 mg) was prepared by a modification of the method of Gilmore and coworkers(17) and freed from excess iodide-iodine by dialysis against buffers containing a trace of thiosulfate and distilled water. Sufficient iodine was used to provide 4 atoms per mole of albumin. Assays for radioactivity were done in a well-type scintillation counter. The starved (24 hr) rabbits used in the washing-type experiments were sedated with morphine, then anesthetized with sodium pentobarbital. An incision was made in the abdomen, and a section of the intestine was removed and cut about 5 inches below the stomach. The apparatus (Fig. 1) was inserted in place about 12 inches below site of the cut. With this arrangement of balloons a section of the intestine was isolated, and the section was continually washed by circulating a solution through it *via* the small diameter polyvinyl tubing. Great care was taken not to damage the intestine during the operation so that no blood appeared in the section being washed. The washing solution used was 1% albumin (either bovine or rabbit) in Krebs-Ringer-bicarbonate buffer at pH 7.8, flowing at a rate of about 1 ml per min. Outflow was collected directly in an equal volume of 20% TCA. When the washing solution had been flowing for 30 min., the albumin-I^{131} or iodide was injected intravenously in amounts indicated in the captions to Fig. 2, 3, 4. Washing solution and blood samples were collected as indicated. Assays for radioactivity were made in the TCA-soluble and insoluble fractions. Small samples of the washing solution, obtained before precipitation with TCA, were tested for blood by the benzidine method. Experiments involving the freezing technic were carried out as follows: Each rabbit was lightly anesthetized and injected with albumin-I^{131} in amounts shown in Tables I and II. The animal was then left undisturbed for an hour, then deeply anesthetized. An incision was made in the abdomen, and a section of the duodenum was gently encircled at each end with 0.25 in. umbilical tape. The 2 pieces of tape were constricted, and albumin solution was injected carefully through a 27 gauge needle till the section was slightly distended. The section was then quickly excised and frozen in a dry ice-acetone bath. The intestinal wall was then peeled off following which the mucosa was scraped off, eventually leaving a frozen core representing contents of the intestinal lumen. After the core was thawed, the albumin solution was centrifuged to remove any remaining particles of mucosa, and solution was precipitated with TCA. Radioactivities were determined as previously noted. Electrophoresis of TCA-insoluble protein was carried out as follows: The precipitate was washed successively with 10% and 1%, TCA, then dissolved in 95% ethanol (18,19). Aliquots of the solution were subjected to zone electrophoresis on paper (Whatman #3 MM) in barbital-barbituric acid at pH 8.6, ionic strentgh 0.05, or pH 6.8, in sodium phosphate buffer 0.025 *M* for 18 hours at 200 volts. After drying, papers were stained with bromphenol blue (0.05% in 1% $HgCl_2$, 2% acetic acid). Then by making use of guide spots of serum albumin, the paper was cut into strips, which were counted in 4 ml of 0.05 *N* NaOH in a vial in the scintillation counter.

Results. Experiments involving washing of sections of the intestines of rabbits injected with either serum albumin-I^{131} or iodine-I^{131} are shown in Fig. 2, 3, 4. When labeled protein was injected a considerable amount of TCA-insoluble, as well as TCA-soluble material, appeared in the intestinal 'excretion' over the whole period of experiments (6.6 and 4 hr, Fig. 2, 3). On the other hand when labeled iodide was injected none appeared in TCA-insoluble form in the intestine (Fig. 4). The activity appearing in TCA-insoluble form could not be accounted for on the basis of blood in the lumen, because the benzidine test remained negative.

When the washing apparatus in the experiment on rabbit #10.0 (Fig. 2) was lowered to a previously unwashed section of intestine, in the following 30 minutes 2 x 10^5 cpm appeared in the washing solution. Of this activity 50% was TCA-insoluble. From this it must be concluded that appearance of activity in the intestine in the first 6.6 hours was

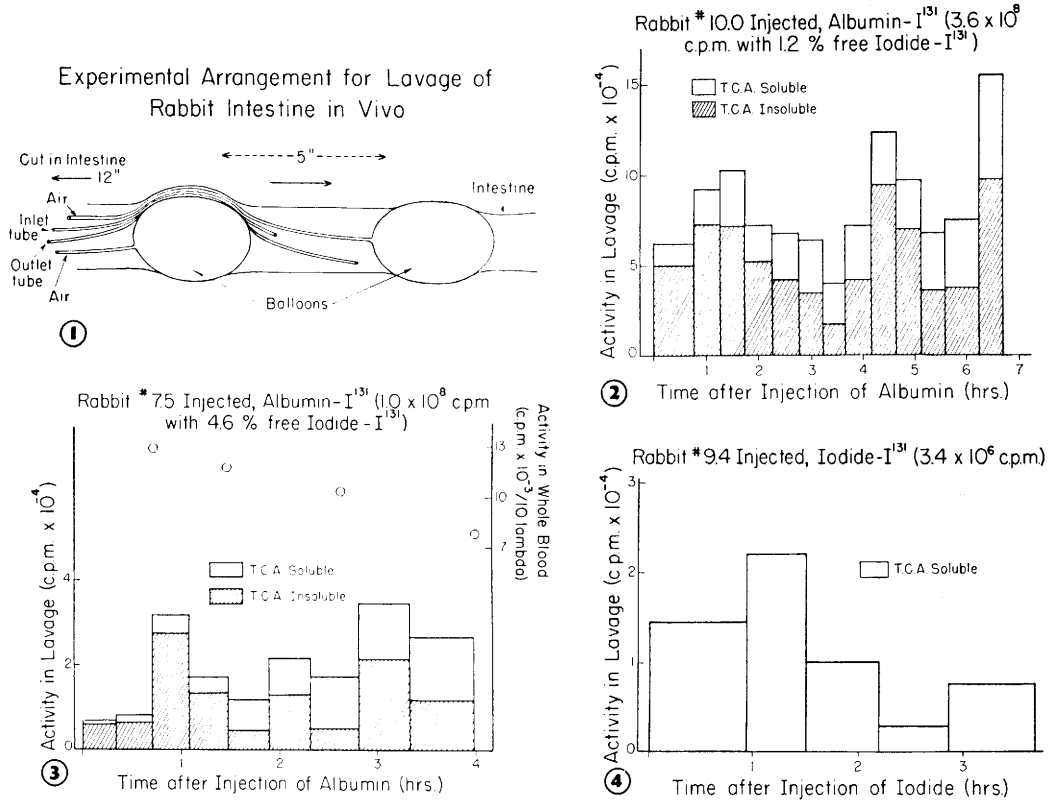

FIG. 1. Experimental arrangement for washing lumen of an isolated section of intestine of rabbits.

FIG. 2. Distribution of radioactivity in washing fluid from lumen of an intestinal loop of rabbit 10 given serum albumin-I^{131} intrav.

FIG. 3. Distribution of radioactivity in washing fluid from lumen of an intestinal loop of rabbit 7.5 given serum albumin-I^{131} intrav.

FIG. 4. Distribution of radioactivity in washing fluid from lumen of an intestinal loop of rabbit 9.4 given iodide-I^{131} intrav.

not due to damage to the tissue.

From these experiments it is possible to make a rough estimate of actual amount of albumin appearing in the section of intestine. In the experiment with rabbit #10 mean specific activity of albumin in the blood over the 4 hour period of experiment was 5.4×10^4 cpm per mg, and mean 'excretion' rate was 10.2×10^4 cpm per hr for TCA-insoluble material. In terms of albumin this represents a minimum excretion of 2 mg per hour for the short section of intestine. In the case of rabbit #7.5 rate was 0.5 mg hr.

If it is assumed that rabbit #10 (4.5 kg) had a total albumin of 13.5 g, with a half-life of 194 hours, then rate of breakdown was 48 mg/hr(20). In other words breakdown in the 13 cm section of intestine represented 4% of the total *in vivo* breakdown. In the rabbit #7.5 (3.4 kg) the observed rate of breakdown in the section was less but still comprised a significant fraction of the total. Therefore, from these experiments it must be concluded that, if excretion occurs elsewhere into the gut as comparable rates, a significant fraction of total albumin broken down by the rabbit may be accounted for by 'leakage' and breakdown in the gut.

The results of experiments carried out by the freezing technic are given in Tables I and II. In these experiments, as in the above, considerable radioactivity was found in the lumen of the intestine of rabbits (#6, 8, 4, 5) injected with albumin-I^{131}. Of this activity found in the lumen about 20% was TCA-insoluble. When this insoluble material

TABLE I. Transfer of Label from Albumin-I^{131} in Serum to Intestinal Lumen (Freezing Technic).

Rabbit	6	8	7*
Dose (cpm × 10^{-8})†	1.8	2.5	.03
Activ. in blood after 1 hr (cpm/2.5 lambda)	1600	604	None
Activ. in lumen (cpm), total × 10^{-4}	11.7	6.4	.06
TCA-insol. (%)	22	15	None

* Rabbit inj. with iodide-I^{131}.
† Free iodide in albumin-I^{131} preparations: Rabbits 6 and 8, 1.5 and 1.6%.

was subjected to electrophoresis, in the 2 cases investigated (rabbits 4 and 5, Table II), about 40% had the mobility and staining properties corresponding to those of serum albumin. About 20% moved from the origin but behaved differently from albumin, and the same per cent remained at origin, probably in the form of denatured protein. The remaining fraction, about 20%, was lost.

These results, although not amenable to quantitative interpretation, show that labeled protein with the properties of albumin appears in the lumen of the intestine of rabbits following injection of labeled albumin into the circulation. Since in these experiments the intestines were subjected to the very minimum of trauma, support is lent to the previous observations when the washing technic was employed. That part of the protein found in the lumen is actually the labeled albumin receives support from the properties of the protein. It is precipitable with TCA, yet TCA-protein is soluble in 95% alcohol, a rather unique property of albumin(18,19), and the protein moves with the same mobility as albumin at 2 pH values, 8.6 and 6.8 in different types of buffered solutions (Table II). When iodide, with radioactivity corresponding to that of the free iodide in the albumin-I^{131} given to rabbit 6, was injected into rabbit 7 no activity appeared in the TCA-insoluble fraction. Thus serum albumin does not acquire any firmly bound radioactivity when iodide-I^{131} is given to rabbits, confirming the results obtained in the washing type experiment with rabbit 9.4 (Fig. 4).

TABLE II. Nature of Label Transferred from Albumin-I^{131} in Serum to Intestinal Lumen (Freezing Technic).

Rabbit	4*	5
Dose (cpm × 10^{-8})‡	3.3	4.8
Length of section (in)	4	6
Total activity in section (cpm × 10^{-4})	14.0	8.9
TCA-insoluble (%)	23	22
Electrophoresis of TCA-insol. activity†		
Origin (%)	26	16
Albumin "	39	40
Remainder "	20	23

* Activity in blood at 1 hr 7,700 cpm/2.5 lambda.
† Zone electrophoresis on paper at pH 8.6. Similar results were obtained at pH 6.8.
‡ Free iodide in albumin-I^{131} preparation: Rabbits 4 and 5, 1.1 and 3.6%.

It should be mentioned that in patients with idiopathic (hypercatabolic) hypoproteinemia there is a great increase in permeability of the gastro-intestinal tract, so that these patients lose the polymer, polyvinyl pyrrolidone (PVP) into the feces when this substance is injected into the blood(15,16), as shown with the iodinated polymer. Subjects with other types of disorders not involving the gastro-intestinal tract were found to lose very little PVP from the circulation into the feces. Although PVP has an average molecular weight of 40,000, it appears possible that the substance does not behave like albumin, and thus might not be lost into the gastro-intestinal tract in normal subjects.

Summary. 1. Experiments have been carried out to show that serum albumin labeled with I^{131} passes from the circulation of rabbits into the duodenum. 2. Following injection of albumin-I^{131} the presence of TCA-insoluble activity in a solution passed through the lumen of isolated section of intestine can be shown. 3. In other experiments of a somewhat different type, the same transfer has been shown. 4. Material transported appears to be albumin since it is precipitated with TCA, the precipitate is soluble in 95% alcohol (like similar precipitate of pure serum albumin), and the soluble material possesses the same electrophoretic mobility as albumin in experiments carried out on paper. 5. Approximate calculations indicate that if this type of transfer occurs in a considerable part of the gastro-intestinal tract, then the enzymatic breakdown of serum albumin so transferred could account for most of the serum albumin breakdown which occurs *in vivo*.

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Etiologic Studies of Trachoma on Taiwan.* (25607)

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The etiologic agent of trachoma has for many years been considered to be a virus of the psittacosis-lymphogranuloma venereum (psitt.-LGV) group. Until recently strains of elementary bodies probably representing viruses of this group which were isolated in the yolk sac of eggs from trachoma patients were lost or not confirmed(1). Recent improvements in technic, particularly incorporation of streptomycin, have resulted in isolation of viruses thought to be the cause of trachoma (2), which have been confirmed(3). The adenovirus group have been shown to be an important cause of eye infections(4) and they are known to be frequent causes of acute conjunctivitis in Taiwan(5,6). Adenoviruses have been isolated from cases of trachoma in Saudi Arabia and even considered as a possible cause of the trachoma syndrome(7,8). It is also possible that adenoviruses may complicate or cause part of the clinical picture or contribute to the spread of trachoma through seasonal acute conjunctivitis. Utilizing the newly perfected technics for yolk sac isolation of elementary body agents and standard tissue culture technics for adenovirus, a study was undertaken of the viral etiology of trachoma on Taiwan.

Materials and methods. During October and November of 1958, specimens were collected from first grade school children in 15 of the 16 counties of Taiwan, including the Pescadore Islands. A total of 1600 eye swabs, throat swabs and blood specimens were collected from children on whom a diagnosis of the condition of their eyes was made by oph-

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