

RATE OF BREAKDOWN AND ABSORPTION OF I^{131} -LABELED CASEIN
IN THE ALIMENTARY TRACT AND OF EXCRETION OF ITS HYDROLYSIS
PRODUCTS WITH THE BILE

K. S. Zamyckina

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In the author's previous investigations of the absorptive power of the alimentary tract of healthy dogs and of dogs after partial resection of the stomach (by Finsterer's modification of the Billroth II method), casein- I^{131} was used as the protein substrate [2, 3]. In these investigations attention was directed to the extremely rapid breakdown of the casein- I^{131} in the alimentary tract. The radioactivity appeared in the blood 10-20 min after administration of the casein- I^{131} to the animals by mouth, mixed with milk.

There are isolated reports in the literature on the use of labeled protein for the study of the rate of its hydrolysis in the alimentary tract [4-6].

In the present investigation the rate of hydrolysis of casein was studied in experiments in vitro and on dogs. The rate of hydrolysis of casein in the alimentary tract and of absorption of the products of its hydrolysis was estimated from the appearance of radioactivity in the blood and bile. Determination of the rate of appearance of the label in the bile after introduction of casein- I^{131} into the alimentary tract, and also the study of the nature of the hydrolysis products of casein- I^{131} excreted in the bile, by the method of chromatography may provide indirect evidence of the rate of breakdown of casein- I^{131} and of the absorption of its hydrolysis products in the alimentary tract.

As the author has previously reported, in dogs with a fistula of the gall bladder (after operations of the Schiff or Schwann type), pathological changes take place in the liver soon after the operation [2, 3].

The present investigation was carried out at different times after formation of a fistula of the gall bladder. Its object was to determine the role of the pathological process arising in the liver and of its bile-forming function in the absorption of casein- I^{131} from the alimentary tract, in the elimination of hydrolysis products of casein- I^{131} not utilized by the organism from the blood, and in their excretion with the bile.

EXPERIMENTAL METHOD

In the experiments in vitro, casein- I^{131} solution with an activity of 5000-10,000 pulses/min/ml was poured in a volume of 1 ml into a series of test tubes. To each tube 2-4 mg of nonlabeled casein and 1 ml of phosphate buffer (pH 7.6) were added, together with a mixture of pancreatic and intestinal juice obtained from dogs with chronic fistulas. The tubes were incubated at 37.5° for 5, 10, 15, 20, and 30 min, and 1, 1.5, 2, or 3 h. After the end of incubation the proteins were precipitated by the addition of an equal volume of 20% trichloroacetic acid solution. The filtrate (0.5 ml) was transferred to a plexiglass target, dried at room temperature, and its radioactivity was measured as β -radiation with an end-type counter. In control experiments the trichloroacetic acid was added soon after the substrate had been mixed with the digestive juices. The radioactivity of the trichloroacetic filtrate was a measure of the degree of hydrolysis of the protein, which was expressed as a percentage.

The nature of the products of hydrolysis of the casein- I^{131} was studied by the paper chromatography method (ascending in a mixture of butyl alcohol and water, 80:20).

In the experiments on the dogs with a gall-bladder fistula of Schiff or Schwann type, casein- I^{131} was given in milk (50 ml) and water (100 ml) in a dose of 100-200 pulses/min/g body weight. To prevent the accumulation of I^{131} in the thyroid, the dogs received a few drops of Lugol's solution mixed with milk daily for 4-5 days. The radioactivity of the samples of blood and bile taken 10 and 30 min, and 1, 1.5, 2, 3, 4, 5, and 24 h after the animals received the casein- I^{131} was determined. The results of the measurements of the radioactivity found in 1 ml of the

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Activity in 1 ml of Blood Serum or Bile Expressed as a Percentage of Activity per Gram Body Weight at Different Times after Operation to Form a Fistula of the Gall Bladder in the Dog Lira

Time of taking blood after administration of casein- I^{131} to animals (in hours)	2 Months 10 days		5 Months		8 Months	
	serum	bile	serum	bile	serum	bile
$1/2$	92	200	98	18	92	14
1	138	500	123	250	130	160
$1\frac{1}{2}$	145	820	126	—	140	200
2	156	1100	160	404	170	260
3	150	1560	163	625	190	450
4	130	2650	197	705	210	500
5	120	3520	190	1000	200	700

test serum and bile were expressed as percentages of the radioactivity per gram body weight. The experiments were carried out on four animals with a fistula of the gall bladder (duration of the experiments 8-10 months) and on three intact dogs.

EXPERIMENTAL RESULTS

The experiments in vitro showed that the hydrolysis of casein- I^{131} by a mixture of pancreatic and intestinal juice takes place very rapidly. Radioactivity was found in the trichloroacetic filtrate after incubation of the protein with the juices for only 2-5 min; during this time 20-25% of the protein was hydrolyzed. After 10-12 min the proportion hydrolyzed was 60-80%, and after 20-30 min, 95-98%.

In the intact dogs, and also in the animals used in the experiment 1-2 months after formation of the gall-bladder fistula, as a result of the administration of casein- I^{131} radioactivity appeared quickly in the blood. The maximal level of radioactivity of the blood was observed after 60-90 min, and its value by the method used for the calculation was 130-160%. Subsequently, the radioactivity of the blood serum gradually fell, and 24 h later it was 30-40%.

After administration of casein- I^{131} to the dogs, intensive excretion of its hydrolysis products in the bile was observed. In samples of bile collected in the first 5-10 min after the animals received the protein, the level of radioactivity per ml of bile was up to 200% of the administered radioactivity per gram body weight. The maximal level of radioactivity of the bile was observed 30-60 min after the dogs received the casein- I^{131} , and it reached 500-700% or more. The high level of radioactivity lasted for a few hours, and after 24 h it had fallen to 10-50%. In the period of development of the pathological process in the liver a slower elimination of the hydrolysis products of casein- I^{131} from the blood was observed. The maximal level of radioactivity of the blood serum was much higher than in the analogous experiments performed on the same dogs in the earlier periods after the operation. At the same time the excretion of the hydrolysis products of casein- I^{131} decreased. In the dog Lira, for example, the activity of the bile 5 h after administration of casein- I^{131} was almost 30 times higher than the activity of the blood. Eight months after the operation the activity of the bile 5 h after the administration of casein- I^{131} was only 3.5 times greater than the activity of the serum (see table).

Similar results were obtained with the other dogs.

The findings, and also reports in the literature, indicate that the amount of bile excreted in the course of the experiment was not always constant. Because of this, the radioactivity was calculated not only per ml, but also in relation to the total volume of bile excreted in a certain time. In these circumstances the same pattern was observed: the excretory function of the liver was depressed as the time after formation of the fistula increased.

The radiochromatographic study of the nature of the hydrolysis products of the labeled casein entering the blood and being excreted in the bile showed that their R_f value was the same, namely 0.23-0.24; the R_f value of inorganic iodine in these same conditions of chromatography is 0.5. Evidently the organic iodine compounds formed during hydrolysis of casein- I^{131} , contained in the blood serum, and excreted in the bile are identical. In these experimental conditions there is no splitting of inorganic iodine (I^{131}) from the casein.

The results of the histological investigation of the liver of the experimental dogs revealed the presence of hepatitis, similar in character to that described earlier by the author and found in dogs with ascending infection of the biliary tract or in dogs after poisoning with carbon tetrachloride [1].

The high radioactivity observed in the blood and bile after administration of casein- I^{131} to the dogs was evidence of the rapid breakdown of the protein in the alimentary tract and of the absorption of its hydrolysis products. This conclusion was also confirmed by the experiments in vitro in which the casein- I^{131} was hydrolyzed by the digestive juices. The bile-forming function of the liver plays an important role in the elimination of the hydrolysis products of casein- I^{131} not utilized by the body. This process is dependent on the functional state of the liver.

The observations described may serve as the basis for the development of diagnostic tests using substances more closely related to the living organism than labeled dyes.

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