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TOXICITY OF HALOGENATED OXYQUINOLINES IN DOGS. A CLINICAL STUDY

IV. TISSUE DISTRIBUTION AND ELIMINATION IN URINE*

By

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LANNEK, BIRGITTA and PAUL LINDBERG: *Toxicity of halogenated oxyquinolines in dogs. A clinical study. IV. Tissue distribution and elimination in urine.* Acta vet. scand. 1974, *15*, 419—435. — Distribution studies using ^{125}I-labelled vioform given orally showed higher blood activity when fat was ingested. It is assumed that this is due to increased absorption from the gastrointestinal tract. Total activity eliminated in urine during 12 hrs. was well correlated with blood activity.

Distribution of activity in blood and tissues in dogs which were intoxicated and died did not show any significant traits as compared to dogs which remained healthy. The mortality was highest in dogs which were fed with fouled herring before the administration of fat and vioform.

v i o f o r m t o x i c i t y; h a l o g e n a t e d o x y q u i n o l i n e s.

Intoxications in dogs following the treatment with halogenated oxyquinolines have been published (*Hangartner* 1965, *Schantz & Wikström* 1965, *Müller* 1967, *Püschner & Fankhauser* 1969, *Lannek* 1973, 1974). The drugs are normally well tolerated which has been ascribed to a supposedly minimal absorption from the gut. They are extremely insoluble in water but moderately soluble in lipid solvents. It was concluded that the absorption was greatly favoured by the contemporary administration of fat (*Lannek & Lindberg* 1972).

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Systemic absorption does take place, however, and part of the substances are excreted in the urine as glucuronic and sulphuric acid conjugates (*Palm* 1932, *Haskins & Luttermoser* 1953, *Ritter & Jermann* 1966, *Berggren & Hansson* 1968).

Suspensions of ^{125}I-labelled vioform (ICOQ, clioquinol, 5-chloro-7-iodo-8-hydroxyquinoline) were given orally to dogs, and the activity was estimated in blood, other fluids, and tissues. Clinical effects have been reported (*Lannek & Lindberg* 1974).

MATERIAL AND METHODS

Animals used in the present investigation have been described (*Lannek & Lindberg* 1974). Altogether 49 dogs, 35 beagles and 14 mongrels, were used in 27 experiments. Twenty-two dogs were males, 27 females. The body weight varied between 7 and 15 kg. Dogs, which did not fall ill during the experiments, and dogs which fell ill slightly, but recovered, were used 2 or more times. The interval was then at least 2 weeks, mostly several months.

ICOQ was given as a suspension through a stomach tube (*Lannek & Lindberg* 1974). The thyroid gland was blocked by giving 2 g potassium iodide 5 days before the experiment (4 dogs), or thiouracile 2×100 mg daily for 2 weeks before the experiment (2 dogs).

The dogs had been starved 12—24 hrs. unless otherwise noted. Other dogs consumed a meal within 1 hr. before the administration of ICOQ suspension (*Lannek & Lindberg* 1974). The food was a commercial preparation (Doggy, Vårgårda) containing about 8 g fat, 20 g protein and 400 cal. per 100 g. In some starved dogs a fat emulsion (Intralipid®, Vitrum), fructose, gelatin or water (to extend the stomach) was given with the ICOQ suspension. The amount of fat emulsion was 80 cal. (40 ml) per kg body weight in 8 dogs and 20—40 cal. (10—20 ml) per kg in 6 dogs. (The quantity had to be decreased, because the large amount will sometimes provoke vomiting). The amount of fructose was 80 cal. in 40 ml water per kg, of gelatin 20 cal. in 40 ml water per kg, and of water 40 ml per kg.

In 2 dogs the fat emulsion was administered as an intravenous drop infusion (20 cal. per kg, 1 drop per sec.) for 2 hrs., starting when the ICOQ was given orally.

The preparation and administration of fouled herring has been described (*Lannek & Lindberg* 1974). It was fed 1—4 days before the ICOQ was given.

Collection of urine

The bladder was emptied by catheterization. A PVC catheter (Willy Rüsch) was introduced and connected to a plastic bag (Urobag, Stille) for collection of the urine. Catheter and bag were fixed to the skin by suturation. Female dogs were slightly sedated and placed on their right side on a table. They were offered water once an hour. The males were left standing on the floor. They had water freely available. A permanent cannula was introduced into the V. antebrachii for obtaining blood samples. Before starting the collection of urine the abdomen was compressed in order to empty the bladder. Bags were exchanged with intervals of 1—4 hrs.

For studies on the distribution of radioactivity tissue samples were collected partly in dogs which became sick and died, and partly in dogs which were killed. In dogs which died, the tissue samples were obtained as soon as possible after death, usually within an hour. Dogs were euthanized by intravenous injection of pentobarbital. No dogs were bled with the exception of Mal., where retrograde aortic perfusion was done (*Lannek & Jönsson* 1974).

Synthesis of ^{125}I-labelled ICOQ (125-ICOQ)

5-chloro-7-iodo-8-hydroxyquinoline was synthetized by chlorination of 8-hydroxyquinoline with sulphuryl chloride to 5-chloro-8-hydroxyquinoline (*Sen-Gupta* 1945). Iodination of 5-chloro-8-hydroxyquinoline was done as described by *Haskins et al.* (1950). In thin-layer chromatographic analyses on polyamide plates and methanol as mobile phase (*Korzun et al.* 1964) each compound showed only 1 distinct spot under u. v. light, or after spraying with Pauly reagent. Rf-values for 8-hydroxyquinoline and its derivatives were comparable with corresponding values of *Korzun et al.* For preparation of 125-ICOQ carrier free sodium iodide (IMS. 3, Radiochemical Centre, Amersham) was used. The radiochemical yield varied between 85 and 95 %, and the specific activities varied from 5.4 to 6.8 μCi/mg. Radioacti-

vity was measured in a gamma counting system with a well-type scintillation detector. Standard deviation of sample counting rate, calculated according to *Comar* (1955), varied from 0.3 to 6.0 %. The doses of 125-ICOQ given orally to dogs varied between 40 and 80 μCi per kg body weight. Carrier ICOQ was used in varying amounts.

Chemical determination of ICOQ in urine

Free ICOQ was determined spectrophotometrically as the iron complex at 650 nm (*Haskins & Luttermoser* 1951). Non-conjugated ICOQ was extracted from 2—5 ml fresh urine with 3×5 ml ethyl ether. The ether phase was washed with 3×2 ml water and dried with anhydrous Na_2SO_4. After evaporation the residue was dissolved in methyl cellosolve, and ICOQ was determined as above. For determination of ICOQ glucuronide 2 ml urine + 2 ml 0.1 M sodium acetate buffer, pH 4.5 + 2 drops β-glucuronidase enzyme (Helix pomatia extract) were incubated for 4 hrs. at 37°C. Released ICOQ was extracted with 3×5 ml ethyl ether. ICOQ sulphate was determined by incubating 2 ml urine + 2 ml H_2O + 0.5 ml concentrated HCl for 1 min. in boiling water, followed by cooling in tap water. Released ICOQ was extracted as above (*Ritter & Jermann* 1966). ICOQ metabolites in urine were estimated by thin-layer chromatography. One ml urine was extracted with 4×3 ml H_2O-saturated n-butanol. The solvent was evaporated under nitrogen. The residue was dissolved in 2 ml methanol. Fifty μl was applicated on a polyamide plate, mobile phase 60 ml n-butanol, 60 ml 0.5 M ammonia, 6 ml ethanol and 3 ml H_2O. After running, 1 cm zones were scraped off and ^{125}I-activity was measured. — Total serum lipids were estimated according to *de La Huerga et al.* (1953).

RESULTS

Tissue distribution

The results of blood activity studies are shown in Tables 1 and 2. Groups of dogs, differently treated, are grossly arranged in rising order of blood activity. Dogs in which the thyroid gland was blocked either by potassium iodide or by thiouracile, show low activity. Starved dogs, in which the thyroid

Table 1. Vioform in blood of dogs which were given ICOQ, including 125-ICOQ, orally (upper row μg/ml, lower row % of dose/ml, for dose 100 mg per kg body weight these values are identical). Figures are mean ± s, or (when number of dogs < 3) individual values. Number of dogs within brackets.

Treatment	Sick/n	Dose ICOQ mg/kg body weight	Hours				
			3	6	9	12	24
thyroid blocked	0/6	100	1.3±0.6 (6)	1.5±0.7 (6)	1.7±0.7 (6)	2.1±0.8 (6)	—
starved	0/1	50	2.1	2.1	1.8	1.7	1.1
			4.2	4.2	3.6	3.4	2.2
„	0/4	100	3.4±2.3 (4)	5.1±4.3 (4)	5.3±5.0 (4)	6.4±5.0 (4)	5.1;8.7
fat emuls. i.v. +							
starved	0/2	100	3.7;1.6	4.9;1.8	—	—	—
water	0/2	100	3.1;1.7	3.9;2.0	2.8;2.3	3.1;3.2	—
fructose	0/2	100	3.2;1.7	3.3;2.6	4.8;2.3	5.2;2.0	—
gelatin	0/1	100	1.9	2.2	4.4	3.5	—
food	0/1	50	8.1	5.6	3.2	—	3.5
			16.2	11.2	6.4	—	7.0
„	1/8	100	16.9±2.6 (8)	12.9±3.8 (8)	9.8±2.5 (5)	7.9;5.6	6.2±1.0 (3)
fat orally	1/2	50	5.5;12.6	7.9;14.8	10.5;9.6	8.4;7.7	5.9;4.1
			11.0;25.2	15.8;29.6	21.0;19.2	16.8;15.4	11.8;8.2
„	0/4	100	9.0±5.9 (4)	14.5±8.9 (4)	17.7±9.5 (4)	13.0±3.3 (4)	6.7;11.7
„	3/4*	300	37.8±2.5 (4)	41.8±7.8 (4)	40.1±4.9 (4)	38.8±6.3 (4)	34.3±6.4 (4)
			12.6±0.8	13.9±2.6	13.7±1.4	12.9±2.1	11.2±2.3
„	3/4	500	27.6±5.9 (4)	32.2±10.9 (4)	33.9±18.8 (4)	30.5±10.6 (4)	24.9±10.5 (4)
			5.5±1.2	6.7±2.3	6.8±3.8	6.1±2.1	5.0±2.1
fat + fouled herring	5/12**	50	8.6±3.5 (9)	9.5±4.9 (12)	8.5±4.3 (12)	8.8±4.4 (4)	7.0±3.4 (8)
			17.2±7.1	19±9.9	17.1±8.5	17.5±8.8	13.9±6.8
„	4/11*	100	17.5±3.4 (7)	17.2±5.9 (11)	15.4±4.7 (11)	17.2±3.6 (5)	15.8±3.9 (10)
„	1/1	300	45.6	32.0	27.5	—	21.3
			15.2	10.7	9.2	—	7.1

* One dog died. ** Two dogs died.

Table 2. Vioform in blood of individual dogs where determinations extended beyond 24 hrs. (upper row µg/ml, lower row % of dose/ml, for dose 100 mg per kg body weight these values are identical).

Treatment	Dog	Dose ICOQ mg/kg body weight	Hours							
			3	6	9	12	24	48	96	120
fat orally	Un.*	50	5.5	7.9	10.5	8.4	5.9	3.5	0.7	0.4
			11.0	15.8	21.0	16.8	11.8	7.0	1.4	0.8
„	Ma.	100	3.3	5.0	7.4	12.8	11.7	10.2	2.4	0.9
„	Fi.*	500	31.1	44.0	59.1	41.4	40.0	25.6	32.8	25.8
			6.2	8.8	11.8	8.3	8.0	5.1	6.6	5.2
„	Mo.	500	24.9	35.0	35.8	34.3	21.4	10.0	2.5	1.0
			5.0	7.0	7.2	6.9	4.3	2.0	0.5	0.2
fat + fouled herring	Al.*	50	9.9	9.0	6.4	4.1	—	1.3	—	—
			19.8	18.0	12.8	8.2	—	2.6	—	—
„	Em.	50	—	19.9	15.2	14.1	—	13.9	—	—
			—	39.8	30.4	28.2	—	27.8	—	—
„	Mal.**	50	6.3	5.5	5.5	—	5.7	—	0.1	—
			12.6	11.0	11.0	—	11.4	—	0.2	—
„	Mi.	100	15.9	12.4	12.1	13.5	—	4.8	—	—
„	Un.*	300	45.0	32.0	27.5	—	21.3	9.1	—	—
			15.2	10.7	9.2	—	7.1	3.0	—	—

* Became sick. ** Became sick and died.

gland was not blocked, show no significant increase as compared with the former group. Starved dogs, which received a fat emulsion intravenously, are on the same blood level as dogs which were only starved. The same is true for dogs given water, fructose or gelatin orally. In dogs given food, fat, or fat + fouled herring, there is a marked increase of blood activity. In the food group, activity is high only initially, however, and falls rather steeply to reach the same level as starved dogs after 10 to 24 hrs. In the fat group and especially in the fat + fouled herring group, the activity remains high up to 24 hrs.

The highest levels, expressed as µg/ml blood, were obtained by the 300 mg/kg dose. A further increase of the dose (500 mg/kg in the fat group) did not result in higher blood activity, but when the result is expressed as % of dose per ml in a fall to approximately half of that obtained by the 300 mg dose. Levels, given as % of dose per ml, seem to be rather constant for the doses 50, 100 and 300 mg. When calculated as µg/ml, they rise linearily with increasing doses.

Table 3. Elimination of 125-ICOQ in the urine. Coefficient of correlation between blood activity and amount eliminated is 0.87.

Treatment	Dog (sex)	Body weight kg	Dose ICOQ mg/kg body weight	Blood activity % of dose/ml (m. + s) (Number of observations)*	Total urine activity/ 12 hrs. % of dose	Urine, ml/12 hrs.
thyroid	Pr. (m)	14	100	2.60±0.56 (4)	6.36	244
blocked	Kl. (m)	15	100	1.75±0.38 (4)	2.01	504
	Mi. (m)	12	100	1.76±0.48 (11)	2.73	766
	Al. (m)	11	100	0.80±0.17 (11)	1.38	384
	Em. (f)	9	100	1.38±0.61 (11)	1.96	478
	Ja. (f)	10	100	1.34±0.64 (11)	2.34	419
starved	A. (m)**	7	100	10.25±3.39 (8)	6.63	104
	B. (f)	9	50	4.25±1.32 (8)	5.17	56
	C. (f)	9	100	4.17±0.85 (8)	4.71	86
	Fim. (f)	10	100	3.64±1.45 (11)	4.60	721
food	Pet. (m)	7	100	7.45±2.26 (11)	11.33	147
	Sl. (m)***	10	100	11.42±3.44 (5)	14.50****	82

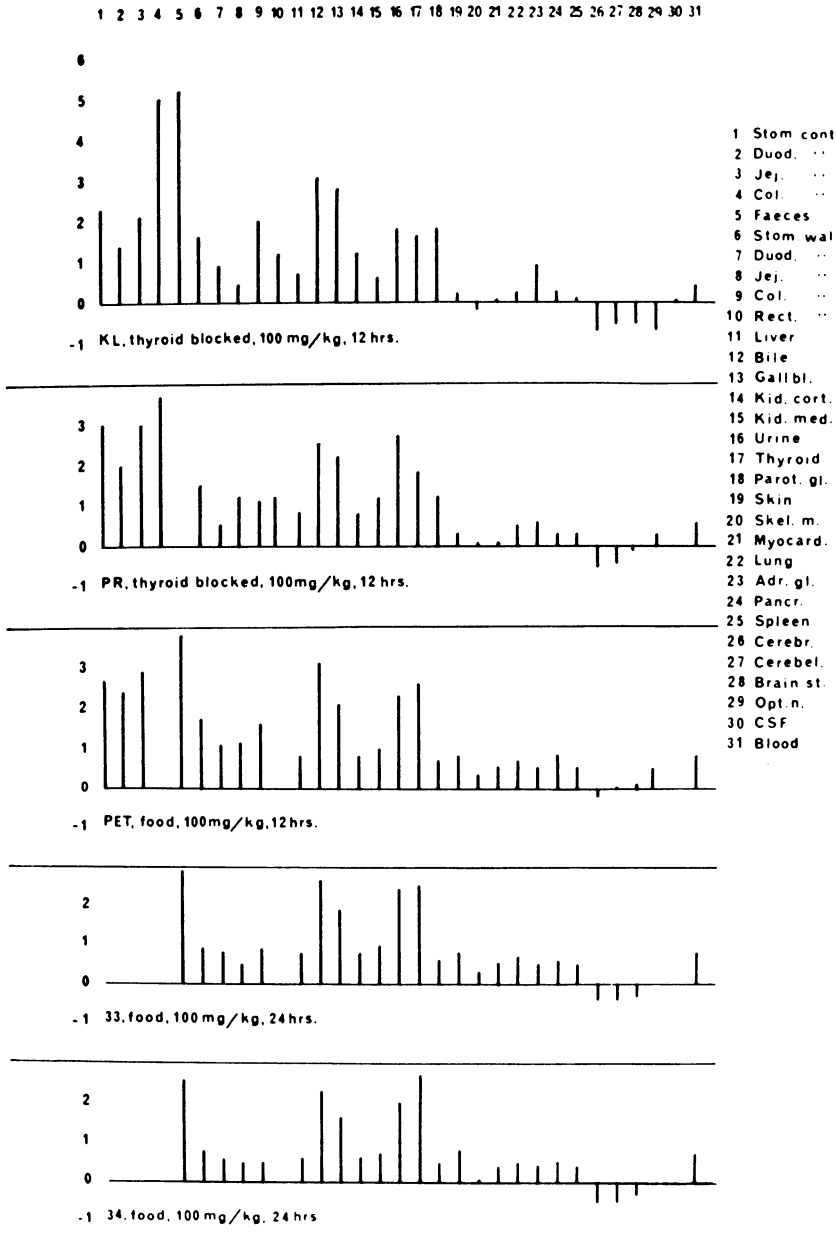
* Mean of observations from 2 to 12 hrs. *** Sick, died.
** Sick. **** In 10 hrs.

Determinations were extended beyond 24 hrs. in some dogs (Table 2). The general impression is that the activity will mostly approach zero at 96 to 120 hrs. An exception is the dog Fi. in the fat group, which still had an activity corresponding to 5.2 % of dose per ml (25.8 µg ICOQ/ml) at 120 hrs. Similarly in the fat + fouled herring group, dog Em. showed only a small decline of blood activity at 48 hrs.

The distribution of activity in other tissues than blood was studied in 10 dogs, which were killed (n = 7) or died (n = 3) (Fig. 1). Three dogs had been starved, and the thyroid gland had been blocked by giving potassium iodide in 2 of them. The others had been given food, fat, or fat + fouled herring. The figures are presented as log. % dose per g tissue.

Elimination in urine

The results are shown in Tables 3 and 4. The amount of 125-ICOQ eliminated during 12 hrs. varied from 1.38 to 6.36 % of


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
KL, thyroid blocked, 100 mg/kg, 12 hrs.
PR, thyroid blocked, 100mg/kg, 12 hrs.
PET, food, 100mg/kg, 12hrs.
33, food, 100 mg/kg, 24hrs.
34, food, 100 mg/kg, 24 hrs
1 Stom cont
2 Duod. ··
3 Jej. ··
4 Col. ··
5 Faeces
6 Stom wall
7 Duod. ··
8 Jej. ··
9 Col. ··
10 Rect. ··
11 Liver
12 Bile
13 Gallbl.
14 Kid. cort.
15 Kid. med.
16 Urine
17 Thyroid
18 Parot. gl.
19 Skin
20 Skel. m.
21 Myocard.
22 Lung
23 Adr. gl.
24 Pancr.
25 Spleen
26 Cerebr.
27 Cerebel.
28 Brain st.
29 Opt.n.
30 CSF
31 Blood

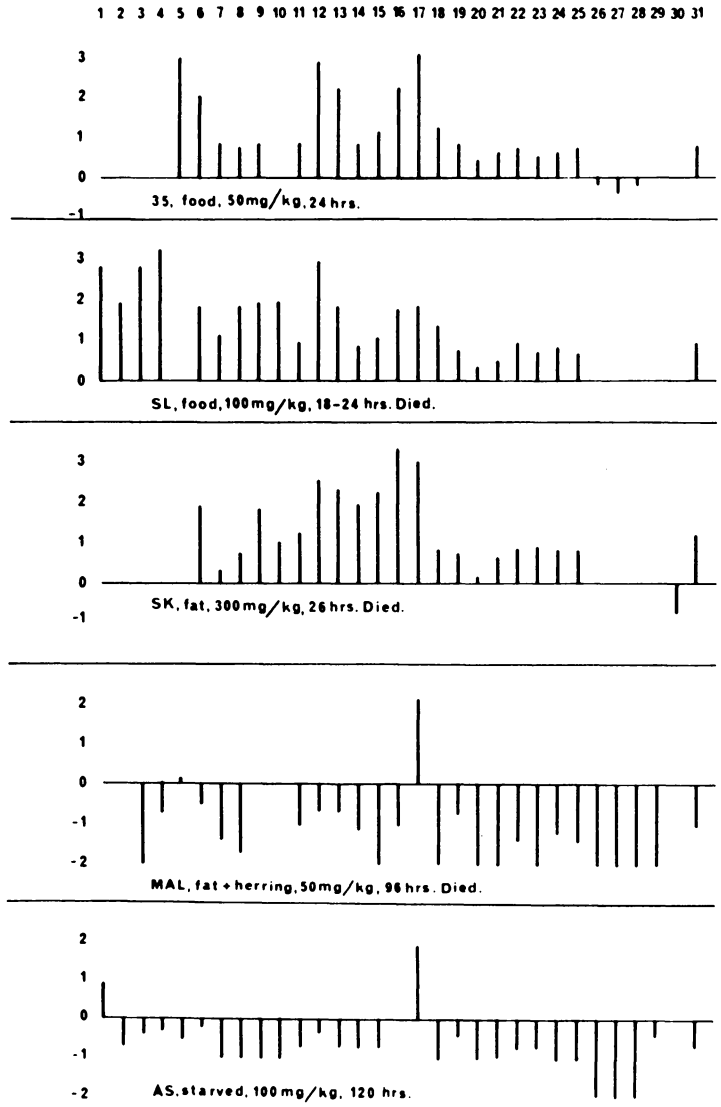


Figure 1. Log.% dose per g of 125-ICOQ in dogs, which were killed or died at 12 to 120 hrs. after oral treatment. The dogs which were killed had not shown any symptoms of disease. The figures are transferred to log. μg/ml by multiplying with 1 (100 mg dose), 0.5 (50 mg dose), or 3 (300 mg dose).

Table 4. Elimination of ICOQ in urine as determined chemically and by radioactivity. The dose was 100 mg/kg except for dog B, which was given 50 mg/kg.

Treatment	Dog (sex)	Urine collected for hrs. 0 —	Volume of urine ml	Chemical determ.		Radioactive determ.	
				mg	% of dose	mg	% of dose
starved	A. (m)	12	104	26.9	3.8	46.2	6.6
	B. (f)	12	56	21.8	5.0	23.0	5.2
	C. (m)	12	86	35.0	4.1	40.2	4.7
	Mi. (m)	6	212	14.7	1.1	21.8	1.7
	Fim. (f)	6	303	21.8	2.2	28.8	2.9
food	Pet. (m)	6	147	58.8	10.3	64.2	11.3
	Sl. (m) *	10	82	153.9	15.4	144.9	14.5

* Sick, died.

the dose in the thyroid blocked dogs, from 4.60 to 6.75 % in the starved dogs, and from 11.73 to 14.50 % in the dogs, which had not been starved (Table 3). There is no relationship between amount eliminated and quantity of urine obtained. The same was apparent when different aliquots of urine of one and the same dog were compared. Table 4 shows the total amounts of ICOQ eliminated in urine as determined chemically or calculated from ^{125}I-activity. Table 5 shows the partition of radioactivity between ether phase and water phase after β-glucuronidase hydrolysis of urine of dogs given 125-ICOQ. The radioactivity distribution of n-butanol extract of urine on polyamide plate is shown in Fig. 2. It is seen that 3 peaks appear. No free ICOQ was detected in fresh urine.

Table 5. Percentage distribution of radioactivity between ether phase and water phase after β-glucuronidase hydrolysis of urine of dogs given 125-ICOQ.

Ether phase	Water phase
46.3	53.7
44.4	55.6
46.9	53.1
38.9	61.1
33.1	66.9

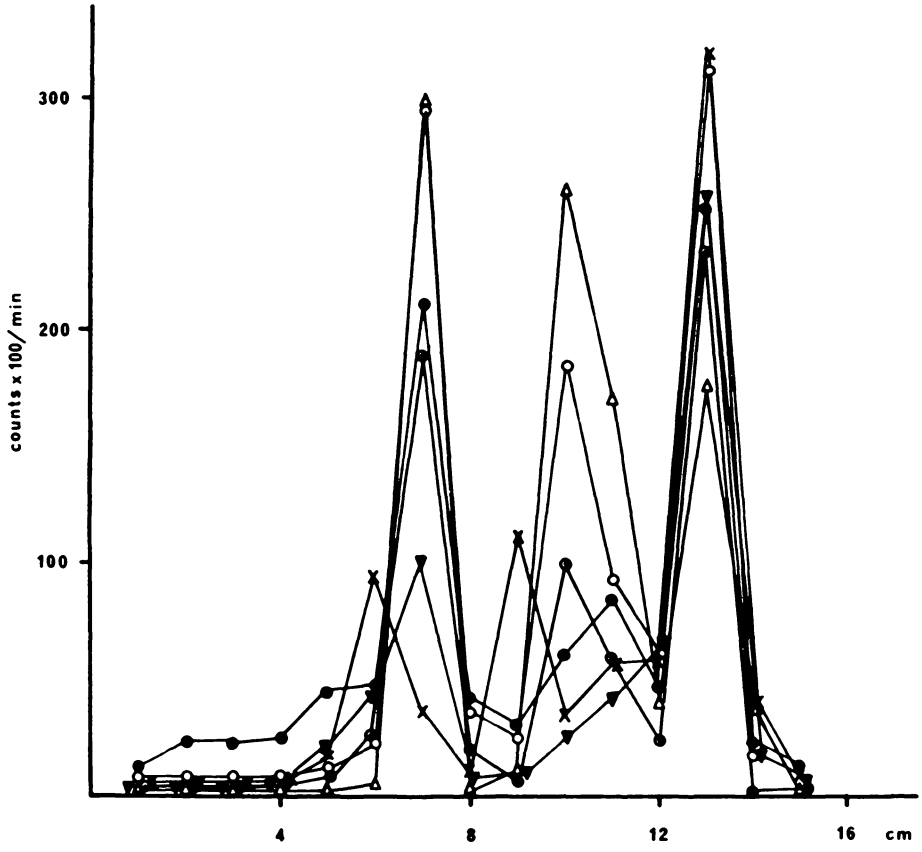


Figure 2. Radioactivity distribution of n-butanol extract of urine of 6 dogs on polyamide plate. Three peaks are visible, Rf-values 0.5, 0.7 and 0.9, respectively.

DISCUSSION

Most of an ingested dose of ICOQ probably passes through the intestinal tract without being absorbed (*Goodman & Gilman* 1971). The therapeutic effect is supposed to be due mainly to the local activity within the bowel. Absorption to some extent does occur, however. This has been confirmed by distribution studies using radioactive components, or by observations of urinary elimination of metabolites, namely glucuronic and sulphate conjugates (*David et al.* 1944, *Knight & Miller* 1949, *Haskins et al.* 1950, *Ritter & Jermann* 1966).

The classical method for estimation of the absorbed proportion of a compound from the gastrointestinal tract is quantitative determination of the excreted part in faeces. This method gives erroneous results, however, if the compound is not only absorbed to but also eliminated from the circulation to the gastrointestinal canal. *Ritter & Jermann* concluded that only minor quantities of ICOQ were absorbed and later excreted in the bile of rats. We did not attempt to measure the bile excretion quantitatively but consistently found high activity in the bile of dogs which had been killed or died (Fig. 1). Species differences probably exist. The radioactivity in blood is a function of absorption, elimination, and probably also reabsorption, and, therefore, need not be closely related to the degree of absorption from the gut. The values presented in Table 1 vary greatly with treatments of the dogs. They are low in starved dogs either the thyroid gland was blocked or not. The simultaneous administration of water, fructose or gelatin does not seem to have had any significant influence. A fat emulsion, however, which was given at the same time as the ICOQ, resulted in much higher blood activity. This was most probably achieved by an enhancement of the absorption through the intestinal wall. The administration of a fat emulsion will produce lipoidemia for several hours. Lipoidemia per se is not associated with high blood radioactivity in dogs given 125-ICOQ. This is apparent from the 2 dogs which were given fat emulsion intravenously for 2 hrs. beginning when ICOQ was given orally. Their blood activity does not exceed that of starved dogs. Similar to the ingestion of fat, the ingestion of food, or fat + fouled herring, is associated with high blood activity. It is concluded that the presence of fats in the intestine is responsible for these effects.

That the high blood activity of these groups is actually a reflection of increased absorption is supported by the increased elimination in urine (Table 3).

It appears (Table 1) that the blood activity expressed as % of dose, is approximately constant, when the doses of ICOQ were 50—300 mg per kg body weight. When the dose is increased to 500 mg/kg, the % absorption is considerably lowered. Some kind of saturation apparently takes place in the dose range 300—500 mg/kg. This is in accordance with observations in rabbits (*Haskins & Luttermoser* 1953).

The activity generally seems to remain on a high level for

longer time in dogs given fat, or fat + fouled herring than in dogs given food. This may be caused by differences in the amounts of fat ingested.

Tissue distribution values (Fig. 1) show rather the same pattern irrespective of treatment. The represented treatment groups are too few, however, to allow a full comparison. Also the time at which the dogs were killed (or died) for tissue distribution studies varies. It ought to be noticed, too, that with 1 exception (Mal.) blood is included in the various tissues. The highest activities expressed as log.% of dose/g seem to be found in the colon content and in faeces. (Some figures representing ingesta are lacking because there were no ingesta to be examined). Also the walls of the gastrointestinal tract are on a high level. The next group in order of activity is excretory organs and excreta, like bile, gall bladder, urine and kidneys.

The thyroid gland obviously has a special position, as its activity may be mainly attributed to absorption of radioiodine arising from breakdown of the 125-ICOQ.

It will be observed that tissues, which have neither an absorbent nor an important excretory role in this connection, like skin, skeletal and heart muscle, lung, pancreas and spleen, show a higher activity in the food-fat group (12—26 hrs.) than in the 2 dogs, which had been starved. Tissue activities of the former dogs are 2—3 times higher than in the latter dogs. This is about the same ratio as for blood.

Blood may under certain conditions constitute a large part of the tissue, e.g. up to 70 % of the forearm skin of man (*Best & Taylor* 1966). It cannot therefore be excluded that the differences are to a significant extent due to the presence of blood. The same discussion applies to brain tissues where the radioactivity is generally low.

At 96—120 hrs. the tissue activity is very low or a fraction of 1 % of dose/g the thyroid gland excepted. An interesting observation is the high activity in the stomach of As. 120 hrs. after the administration. It may possibly originate from saliva which had been swallowed. Saliva (not shown in Fig. 1) contained radioactivity corresponding to about 25 % of the dose/g in 2 sick dogs, which produced saliva in amounts sufficiently large for estimation. No distinct feature of tissue distribution can be noticed in the 2 dogs, which died 18—26 hrs. after the administration.

The radioactivity recovered in urine during 12 hrs. (Table 3) amounts to less than 7 % of the dose in starved dogs. In 2 dogs, which were given food before the administration of 125-ICOQ, the activity is largely doubled. It is noticed that there is a good correlation between mean blood activity and excreted activity.

The data shown in Table 4 imply a close association between ICOQ as determined chemically ("sulphate" + "glucuronide") and estimated from activity. Obviously, the kidneys eliminate mainly bound iodine, which is in accordance with earlier observations (*Haskins et al., Ritter & Jermann*). "Sulphate" and "glucuronide" would thus represent the main bulk eliminated by the kidneys. Three peaks were consistently observed, however, by thin-layer chromatography (Fig. 2). Actually, ICOQ estimated by radioactivity determinations is slightly larger than the sum of "sulphate" and "glucuronide" in 6 dogs out of 7. One of the 3 peaks, which have not been identified, may account for this difference. *Liewendahl et al.* (1967) similarly observed 3 metabolites by paper chromatography of urine from man.

Helix pomatia extract hydrolysis of urine from dogs which had been given ICOQ released only 33—46 % of total ICOQ as free compound in our experiments (Table 5). This is contrary to *Ritter & Jermann,* who obtained 99—100 % by enzymatic hydrolysis of urine from man. *Rodriguez & Close* (1969) showed that an enzymatic mixture of β-glucuronidase and aryl-sulphatase did not release more free 5-7-dibromo-8-hydroxyquinoline from urine of man than a pure β-glucuronidase preparation. These results and our observations indicate that sulphatase activity is inhibited in urine by the presence of sulphate and phosphate ions (*Bradlow* 1970). ICOQ-sulphate is easily split by acid hydrolysis as shown by *Ritter & Jermann.*

It appears (Table 1) that disease and mortality after the administration of ICOQ only occurred in dogs which were given food, fat, or fat + fouled herring. Thus, of 38 dogs which were given 50—100 mg ICOQ per kg body weight 11 fell ill. The factor in common for these groups is the ingestion of fat at the time when they were given ICOQ. In dogs which did not infest fat, the corresponding ratio is 0/18. It should also be observed that the addition of fouled herring to fat seems to have increased the rate of disease at these dose levels or from 2/15 to 9/23. The effect of fat ingestion is probably explained by promotion of absorption. The effect of fouled herring remains obscure so far.

It was the hypothesis that fouled herring contains phenolic compounds, which compete with ICOQ for the coupling to sulphate and glucuronic acid. Although this hypothesis seems to have been supported by the experimental findings, alternative explanations do certainly occur.

ICOQ is supposed to be coupled to sulphate and glucuronide in the intestinal mucosa (*Ritter & Jermann*). ICOQ given intravenously caused immediate disease in dogs (*Lannek & Lindberg* 1974). It may be hypothesised that disease in dogs which have been given ICOQ orally is due to deconjugation in sensitive organs.

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SAMMANFATTNING

Toxiciteten av halogenerade oxikinoliner hos hund. En klinisk studie. IV. Vävnadsdistribution och elimination i urin.

Distributionsundersökningar med ^{125}J-märkt vioform, vilket gavs peroralt, visade högre blodaktivitet, när fett gavs samtidigt. Detta är troligen orsakat av ökad absorption från tarmkanalen. Total aktivitet eliminerad i urinen under 12 timmar var nära korrelerad med blodaktiviteten.

Distributionen av aktivitet i blod och vävnader hos hundar, vilka blev intoxikerade och dog, visade inga signifikanta drag i jämförelse med hundar som förblev friska.

Mortaliteten var högst hos hundar som gavs ruttnad strömming före tillförseln av fett och vioform.

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