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## On glucuronide formation in the cat

(*Received* 8 *April* 1964; *accepted* 19 *May* 1964)

IT HAS been reported[1, 2] and is apparently generally accepted[3, 4] that cats do not use glucuronic acid as a means of detoxifying foreign organic compounds, although they do form a glucuronide of bilirubin.[5] Their failure to use this common mammalian detoxication mechanism has been attributed to a lack of glucuronyl transferase, rather than to an inability to form UDP-glucuronic acid.[6] In man and dog, after the administration of iopanoic acid,* the principal biliary iodine-containing compound has been identified (by isolation) as an ester glucuronide.[7] The same is true[8] of tyropanoic acid.† Iopanoic acid-glucuronide is poorly absorbed when given to cats orally but concentrates in the bile more rapidly than the parent compound when given intravenously.[9] It is also known that cats convert iopanoic acid to a highly water-soluble conjugate.[7] If this conjugate is not a glucuronide, a glycinate seems to be the most likely alternative. To establish which type of conjugation actually occurs the following experiments were performed.

Healthy adult cats weighing 2–3 kg were prepared for study as previously described.[7] The radiopaques (100 mg/kg, calculated as the acids) were administered orally as finely divided powders in capsules (iopanoic acid and its glycine conjugate‡ as the acids; tyropanoate and bunamiodyl§ as the sodium salts). Sixteen hr later the cats were sacrificed under pentobarbital anesthesia, the gall bladders were tied off and removed, and the following items were determined on the bile.

*A. Total iodine* (by the method of Zak and Boyle[10]).

*B. Ether-extractable iodine and conjugated glucuronic acid.* To 0·1–0·2 ml of bile was added 10 ml of 0·1 N hydrochloric acid and 50 ml of ether. After thorough extraction (3–5 min mechanical shaking in 100-ml g.s. centrifuge tubes) and centrifugation, glucuronic acid was determined[11] on the residue from 1–2 ml aliquots of the ether, and total iodine was determined[10] on the residue from 40-ml ether aliquots. (Note: the ether extraction method as described readily extracts the glucuronide conjugates of iopanoic and tyropanic acids[7, 8] but not necessarily quantitatively).

*C. Unchanged radiopaque.* To 0·2 ml of bile was added 10 ml of acetate buffer (0·5 M, pH 4·7), and the solution was thoroughly extracted (as described in *B*, above) with 40 ml of a 1:1 mixture (v/v)

* Available from Winthrop Laboratories, New York, N.Y., under the trade name of Telepaque. Iopanoic acid is 3-amino-α-ethyl-2, 4, 6-triiodohydrocinnamic acid.

† Available from Winthrop Laboratories, New York, N.Y., under the trade name of Bilopaque. Tyropanoic acid is 3-butyrylamino-α-ethyl-2, 4, 6-triiodohydrocinnamic acid.

‡ Prepared by Dr. A. A. Larsen; m.p. 197–198°; % iodine = 61·2.

§ Available from E. Fougera Co., Hicksville, N.Y., under the trade name of Orabilex. Bunamiodyl is 3-butyrylamino-α-ethyl-2, 4, 6-triiodocinnamic acid.

of chloroform and hexane. This procedure extracts at least 95% of unchanged iopanoate, tyropanoate, and bunamiodyl[12] but would not be expected to extract their glucuronic acid esters. It was usually necessary to clarify the extracts by prolonged centrifugation and filtration through sodium sulfate, after which total iodine was determined on 30-ml solvent aliquots.

*D. Radiopaque released by β-glucuronidase.* The same procedure as in *C* was employed, but the solution was incubated for 4 hr at 37° with 150 mg of β-glucuronidase (Nutritional Biochemicals Co., from bovine liver; 1 mg = 65 Fishman units [claim]) prior to the extraction with chloroform–hexane. Iodine in the extract was determined as in *C*[10]. (See also footnote 5 to Table 1.)

*E. Enzymatic digestion control.* The solution was incubated as in *D* but with no enzyme added. Extraction and analysis were carried out as in *C*.

These experiments were performed on 12 cats, 7 of which received iopanoic acid, 2 received sodium tyropanoate, 1 received sodium bunamiodyl, 1 received the iopanoic–glycine conjugate, and 1 was not medicated (in order to supply the blank values for ether-extractable glucuronides). The results are presented in Table 1.

TABLE 1. METABOLISM OF SOME ORAL CHOLECYSTOGRAPHIC AGENTS IN THE CAT AFTER THE ADMINISTRATION OF 100-MG/DOSES PER KG

| | | Item determined [as mg iodine or glucuronic acid (G.A.) per ml of bile][a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | *A* | *B* | | *C* | *D* | *E* |
| Compound given | Cat no. | | $I_2$ | G.A.[c] | | | |
| Iopanoic acid | 1 | 24·4 | 21·7 | 9·8 | 0·1 | 23·0 | 1·9[f] |
| | 2 | 26·4 | 27·1 | 12·5 | 0·5 | 28·2 | 0·5 |
| | 3 | 24·3 | 22·5 | 9·4 | 1·1 | 20·7[d] | 2·8[f] |
| | 4 | 28·0 | 25·8 | 11·4 | 0·1 | 12·0[d] | 0·2 |
| | 5 | 24·3 | 23·4 | 9·9 | 0·1 | 11·3[d]<br>6·8[e] | 0·3 |
| | 6 | 11·3 | 11·1 | 4·0 | | 0·2[e]<br>9·9[d] | 0·3 |
| | 7 | 12·1 | 11·0 | 3·9 | | 0·1[e] | 0·2 |
| Tyropanoate = Na | 8 | 31.2 | 26·5 | 11·0 | 2·5 | 16·9 | 2·5 |
| | 9 | 22·2 | 18·5 | 9·8 | 0·7 | 15·4[d] | 0·7 |
| Bunamiodyl = Na | 10 | 7·9[b] | 7·3 | 2·1 | 1·5 | 4·3 | 1·1 |
| Iopanoic = glycinate | 11 | 15·6 | 10·7 | 0 | 5·9 | 2·0 | 5·8 |

[a] For explanation of items *A–E*, see text.

[b] This bile sample was very dilute.

[c] Blank values from the unmedicated cat (1 mg/ml) have been subtracted.

[d] A different lot of glucuronidase was used to digest these samples. It was less potent, since when used to digest the bile samples from cats 2,8, and 10 it released only half as much radiopaque as the original enzyme preparation. The sulfatase activity of the former was equivalent to 8% of its glucuronidase activity (based on comparative rate of release of *p*-nitrophenol per hr at pH 5·5, 37°).

[e] Taka-diastase (Parke-Davis), 6 g., was used to digest these samples. This enzyme had no β-glucuronidase activity but had sulfatase activity equivalent to the glucuronidase activity of the NBC enzyme used to digest the same samples. Recovery of iopanoate added to this enzymatic digest was 71%.

[f] It was not possible to clarify these extracts completely.

## COMMENT

If the radiopaques were completely converted to ester glucuronides there should be present in the bile 0·51 mg of extra glucuronic acid for each mg of organic iodine. The percentage of unchanged radiopaque in the bile is indicated by the relationship of the amount of iodine in item *C* (or *E*) to that in *A*. This varied from 2% (mean) for iopanoate to 19% for bunamiodyl (one experiment). On this basis there should be 0·5 mg of extra glucuronic acid for each mg of iodine in the iopanoate biles, 0·47 to 0·49 mg in the tyropanoate biles, and 0·41 mg in the bunamiodyl bile. However, glucuronic acid was determined, not on the whole biles, but on ether extracts thereof. The ratio of glucuronic

acid to iodine in the ether extracts (corrected for the amounts of unconjugated radiopaques) varied from 0·36 to 0·47 for iopanoate (mean: 0·42), from 0·46 to 0·55 for tyropanoate, and was 0·36 for bunamiodyl. Further evidence that glucuronide formation occurs with all three radiopaques is their liberation by β-glucuronidase (*D–E*, as compared to *A–C*). This averaged 71% of completion for iopanoate, 57% for tyropanoate, and was 50% for bunamiodyl. An enzyme preparation containing sulfatase but no glucuronidase (cats 6, 7) released no iopanoate from conjugation.

The solvent-distribution characteristics of the iodine-containing compound eliminated in the bile after administration of the glycine conjugate of iopanoic acid (cat 11) suggest that this compound is not excreted unchanged since, when the known conjugate was added in an equivalent amount to bile from the unmedicated cat, it was found to be completely extracted by ether (*B*), and 65% extracted by the chloroform–hexane mixture (*C*).

*Acknowledgment*—The author is indebted to Sandra Regina and John Romano for technical assistance.

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