

would expect some day-to-day variation. Paterson and Lawrence (1972), in a study of 42 women, concluded that this was so. We too have found considerable day-to-day variation in healthy adults. The values tended to fall during the first part of the week, perhaps a consequence of the relatively sedentary nature of hospital work after the activity of the weekend. Marked increases could usually be related to increased exercise—for example, squash playing. The day-to-day variation is obviously important in assessing the significance of rises of serum CK from any cause. We have calculated that an increase should exceed 85% of the initial value to be significantly greater than variations seen in normally active adults. This criterion of significance would therefore tend to underestimate the significance of increases in patients at rest in bed.

The quality control data shows that with our manual method single estimations of enzyme activity have a coefficient of variation of 18% at 67 IU/l. and 13% at 524 IU/l. Thus, analytical variation makes a significant contribution to our criterion of significance. Future improved analytical methods will probably allow a smaller change in enzyme activity to be recognized as significant.

Though intramuscular injections might confuse the interpretation of serum CK (Hess *et al.*, 1964; Cohen, 1972; Goldberg and Winfield, 1972) a review of the literature shows that very few drugs have been adequately studied. Marked increases of serum CK in six out of 14 patients after single injections of chlorpromazine have been well documented by Meltzer *et al.* (1970), who also showed increases in both subjects given an injection of phenobarbital. Fässler and Vorburger (1973) in a study of 24 subjects stated that "significant rises" of serum CK resulted from injection of diazepam and pentazocine, but details were not given of the magnitude or frequency of such rises. Batsakis *et al.* (1968) and Hess *et al.* (1967) found raised serum CK values in patients given frequent injections of penicillin, but no preinjection values were given. The diagnoses were not stated except in one patient who had pneumonia, a reported cause of raised CK values (Vélez-García, 1966; Perkoff, 1968). Batsakis *et al.* (1968) also found raised values in four subjects receiving four-hourly injections of morphine, but no preinjection values were given and again the diagnoses were not stated. Chahine *et al.* (1974) showed that increases in serum CK after cardiac catheterization could be related to prior intramuscular injections of various drugs, but the effects of the individual drugs were not assessed.

We found that single injections of diamorphine and pentazocine cause significant increases of serum CK values in about a quarter of patients (three out of 15 given pentazocine; three

out of 10 given diamorphine). The greatest increase was from 64 IU to 395 IU/l. These two drugs are the analgesics most commonly used for myocardial infarction in this hospital and the increase in serum CK associated with their use could cause confusion in diagnosis. We suggest that when possible blood should be collected before the first injection to detect any early diagnostic increase of serum CK. Any subsequent rise must be interpreted with caution.

Despite the effects of exercise and of intramuscular injections on serum CK values, Goldberg and Winfield (1972) showed that serum CK was the most reliable single enzyme test for the diagnosis of recent myocardial infarction. In the future it may be possible to measure the cardiac isoenzyme of CK routinely (Kontinen and Somer, 1973; Mercer, 1974). Meanwhile, consideration of the effects of exercise and injections along the lines we have suggested should lead to greater accuracy in diagnosis.

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References

- Batsakis, J. G., *et al.* (1968). *Clinical Biochemistry*, 2, 125.
 Campbell, D. G., and Owen, J. A. (1967). *Clinical Biochemistry*, 1, 3.
 Chahine, R. A., Eber, L. M., and Kattus, A. A. (1974). *American Heart Journal*, 87, 170.
 Cohen, L. (1972). *Journal of the American Medical Association*, 219, 625.
 Dalal, F. R., Cilley, J., jun., and Winsten, S. (1972). *Clinical Chemistry*, 18, 330.
 Dubo, H., *et al.* (1967). *Lancet*, 2, 743.
 Ebashi, S. L., *et al.* (1959). *Journal of Biochemistry*, 46, 103.
 Fässler, B., and Vorburger, C. (1973). *Schweizerische medizinische Wochenschrift*, 103, 311.
 Goldberg, D. M., and Winfield, D. A. (1972). *British Heart Journal*, 34, 597.
 Griffiths, P. D. (1966). *Clinica Chimica Acta*, 13, 413.
 Hess, J. W., *et al.* (1964). *Annals of Internal Medicine*, 61, 1015.
 Hess, J. W., *et al.* (1967). *Clinical Chemistry*, 13, 994.
 Kontinen, A., and Somer, H. (1973). *British Medical Journal*, 1, 386.
 Levell, M. J., and Payne, R. B. (1973). *Diagnostica*, No. 28, p. 10.
 Meltzer, H. Y., Mrozach, S., and Boyer, M. (1970). *American Journal of Medical Sciences*, 259, 42.
 Mercer, D. W. (1974). *Clinical Chemistry*, 20, 36.
 Oliver, I. T. (1955). *Biochemical Journal*, 61, 116.
 Paterson, Y., and Lawrence, E. F. (1972). *Clinica Chimica Acta*, 42, 131.
 Payne, R. B., and Levell, M. J. (1968). *Clinical Chemistry*, 14, 172.
 Perkoff, G. T. (1968). *Archives of Internal Medicine*, 122, 326.
 Savignano, T., Hanok, A., and Kuo, J. (1969). *American Journal of Clinical Pathology*, 51, 76.
 Vejajiva, A., and Teasdale, G. M. (1965). *British Medical Journal*, 1, 1653.
 Vélez-García, E., *et al.* (1966). *Journal of Laboratory and Clinical Medicine*, 68, 636.
 Watkins, S. M., and Lewis, A. (1972). *British Medical Journal*, 3, 733.

Pruritic Effect of Bile Salts

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Summary

The pruritic effect of purified bile salts has been tested by applying them to blister bases. All the salts tested were

pruritogens, but the dihydroxy salts (especially unconjugated chenodeoxycholate) were more effective than the trihydroxy salts. This may explain the poor correlation between total serum bile salt concentration and pruritus in obstructive jaundice.

Introduction

The pruritus of obstructive jaundice is thought to be due to bile salt retention (Herndon, 1972). Several previous attempts to induce itching by applying bile salts to the skin have been unsuccessful, however (Brulé and Cottet, 1942; Schoenfeld, 1969), and the correlation between serum bile salt concentration and pruritus is poor (Osborn *et al.*, 1959; Datta and Sherlock, 1963; Neale *et al.*, 1971). It seems likely that the various salts

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might differ in their ability to provoke pruritus, and we therefore tested the pruritic effect of purified bile salts by applying them directly to intact skin and to blister bases. We used the terms "bile salt" and "bile acid" interchangeably though in man the bile salts are mainly conjugated and ionized.

Methods

We tested 10 men and 14 women, aged 18-75 years (mean 45), who either were normal volunteers or had localized non-irritable dermatoses. None of the subjects had ever been jaundiced, and none complained of spontaneous itching.

The nine compounds tested were the purified sodium salts of the three main bile acids and their taurine and glycine conjugates (Maybridge Chemicals, Tintagel): cholate, deoxycholate, chenodeoxycholate, glycocholate, glycodeoxycholate, glycochenodeoxycholate, taurocholate, taurodeoxycholate, and taurochenodeoxycholate.

These compounds were each dissolved in phosphate buffered saline at 1 mmol/l. concentration with the exception of the deoxycholate and chenodeoxycholate, which were insoluble in saline. These two compounds were first dissolved in 5% sodium bicarbonate and then diluted with buffered saline to a bile salt concentration of 1 mmol/l. The pH of all these final solutions was within the range 6.9-7.1. This concentration was chosen because the serum bile salt concentration in obstructive jaundice is about 0.3 mmol/l. (Osborn *et al.*, 1959), and some allowance was made for the possibility of preferential binding of bile salts in the skin (Schoenfield *et al.*, 1967).

Prick and Patch Tests.—In six subjects the most concentrated solutions of each bile salt were applied to the back under an occlusive dressing for 48 hours as conventional patch tests. Each bile salt was also tested by prick tests in the forearm skin in six subjects.

Blister Base Tests.—In 20 subjects the solutions were tested by applying them to the base of cantharidin-induced blisters on the ventral surface of the forearm, by the technique of Arthur and Shelley (1955). The vesicles were produced by the application of 0.2% cantharidin plasters to the skin for six hours on the evening before the experiment, and on the next morning each vesicle was carefully aspirated. Then 0.1 ml of each test solution was injected under a blister roof and was washed out with normal saline after two minutes. Room temperature was constant at about 20°-22°C and other interfering sensory stimuli were kept at a minimum. The subjects were told that some of the solutions might cause an abnormal sensation, and they were asked to describe what they felt at the end of the two-minute test period. As control solutions we used normal saline and papain (Sigma Labs. 16-40 B.A.E.E. units/mg) diluted 1/10. Each solution was tested singly in a double-blind way, and the order of testing was randomized.

Results

The results of the prick tests and patch tests were entirely negative, no pruritus or erythema being produced in any subjects.

In the blister base tests all of the bile salts produced pruritus in at least two patients (see table). The itch developed within 45 seconds of the injection and occurred in "waves" as an abnormal prickling sensation. In some subjects this progressed to a burning or stinging sensation which faded within five minutes of irrigating the blister with saline. The dihydroxy bile salts—deoxycholate, chenodeoxycholate, glycodeoxycholate, glycochenodeoxycholate, taurodeoxycholate, and taurochenodeoxycholate—were more effective in causing pruritus than the trihydroxy salts—cholate, glycocholate, and taurocholate (see table). On pooling these results the trihydroxy salts caused pruritus in 11 out of 60 tests, and the dihydroxy salts produced an itch in 56 out of 120 tests. A χ^2 test showed this to be a highly significant difference ($P < 0.001$), and the difference was greater

Pruritic Effect of Purified Bile Salts when Applied to Blister Bases. Results are Numbers of Subjects who developed Pruritus.

	Free Acids	Taurine Conjugates	Glycine Conjugates
Cholate	4	2	5
Deoxycholate	10	8	6
Chenodeoxycholate	17	9	6

with the unconjugated salts than with the conjugated salts. The control saline injections produced no itching, but the papain caused pruritus in all 20 subjects.

Discussion

Previous attempts to induce itching with exogenous bile salts have been largely unsuccessful. The intravenous (Osborn *et al.*, 1959; Brulé and Cottet, 1942) and oral (Carey, 1958) administration of cholate failed to produce an itch though crude bile salt ingestion did exacerbate pruritus in patients with biliary cirrhosis (Ahrens *et al.*, 1950). Topical application (Brulé and Cottet, 1942; Schoenfield, 1969) and iontophoresis (Schoenfield, 1969) of bile salts into the skin have also failed to produce pruritus. Varadi (1974), however, has reported that crude bile when applied to keratin-stripped skin under occlusion at acid pH will cause pruritus though he found no pruritus was produced at neutral pH. Our present observations show that purified bile salts at a concentration of 1 mmol/l. can cause pruritus in human skin at neutral pH, and the dihydroxy salts (particularly unconjugated chenodeoxycholate) are more effective pruritogens than the trihydroxy salts. Varadi (1974) also observed that chenodeoxycholate at acid pH was more effective than cholate in producing pruritus. The poor correlation between serum bile salt concentration and pruritus (Osborn *et al.*, 1959; Datta and Sherlock, 1963; Neale *et al.*, 1971) may thus be due to variation in bile salt composition. In complete cholestasis the deoxycholates, being secondary bile acids, disappear, the proportion of glycine-conjugated salts decreases, and the ratio of chenodeoxycholate to cholate tends to increase (Neale *et al.*, 1971). Cholestyramine, which relieves the pruritus of obstructive jaundice (Carey and Williams, 1961), has been shown to bind dihydroxy salts preferentially (Johns and Bates, 1969, 1970). Changes in the relative proportion of dihydroxy salts such as unconjugated chenodeoxycholate might thus account for the discrepancy between total circulating bile salt concentration and pruritus. Bile salts have been recovered from the skin surface of jaundiced patients with pruritus (Schoenfield *et al.*, 1967), and 85% of the recovered bile salts were in the unconjugated form.

The mechanism by which bile salts cause pruritus remains speculative. Unconjugated bile salts are toxic to a variety of tissue and cell preparations *in vitro*, and this non-specific cytotoxicity is thought to relate to their detergent effect on lipid membranes (Heaton, 1972). The dihydroxy salts are more toxic than the trihydroxy salts, and this increased toxicity correlates with their increased surface activity (Dreher *et al.*, 1967). Chemical mediators of itching may act by directly depolarizing cutaneous nerves or by releasing endogenous pruritogens such as histamine or proteolytic enzymes. Bile salts liberate small amounts of histamine on perfusion of animal skin (Schachter, 1952), and blood histamine levels increase in experimental obstructive jaundice (Anrep and Barsoum, 1953), but the poor correlation between pruritus and plasma histamine in liver disease (Mitchell *et al.*, 1954) and the poor therapeutic response to antihistamines suggests that histamine is not the major mediator of pruritus.

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References

- Ahrens, E. H., Jun., *et al.* (1950). *Medicine*, 29, 299.
 Anrep, G. V., and Barsoum, G. S. (1953). *Journal of Physiology*, 120, 427.
 Arthur, R. P., and Shelley, W. B. (1955). *Journal of Investigative Dermatology*, 25, 341.
 Brulé, M., and Cottet, J. (1942). *La Presse Médicale*, 50, 369.
 Carey, J. B. (1958). *Journal of Clinical Investigation*, 37, 1494.
 Carey, J. B., and Williams, G. (1961). *Journal of the American Medical Association*, 176, 432.
 Datta, D. V., and Sherlock, S. (1963). *British Medical Journal*, 1, 216.
 Dreher, K. D., Schulman, J. H., and Hofmann, A. F. (1967). *Journal of Colloid and Interface Science*, 25, 71.
 Heaton, K. W. (1972). *Bile Salts in Health and Disease*, p. 117. London, Churchill Livingstone.
 Herndon, J. J. (1972). *Archives of Internal Medicine*, 130, 632.
 Johns, W. H., and Bates, T. R. (1969). *Journal of Pharmaceutical Sciences*, 58, 179.
 Johns, W. H., and Bates, T. R. (1970). *Journal of Pharmaceutical Sciences*, 59, 329.
 Mitchell, R. G., Butt, H. R., and Code, C. F. (1954). *Journal of Clinical Investigation*, 33, 1199.
 Neale, G., *et al.* (1971). *Gut*, 12, 145.
 Osborn, E. C., *et al.* (1959). *Lancet*, 2, 1049.
 Schachter, M. (1952). *British Journal of Pharmacology*, 7, 646.
 Schoenfeld, L. J. (1969). In *Bile Salt Metabolism*, ed. L. Schiff, p. 257. Springfield, Thomas.
 Schoenfeld, L. J., Sjoval, J., and Perman, E. (1967). *Nature*, 213, 93.
 Varadi, D. P. (1974). *Archives of Dermatology*, 109, 678.

Neonatal Hyperthyroidism and Long-acting Thyroid Stimulator Protector

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Summary

Neonatal hyperthyroidism has been thought to result from transplacental passage of long-acting thyroid stimulator (L.A.T.S.) from a mother with Graves's disease. A case is presented here in which no L.A.T.S. was detected in the mother or neonate but another immunoglobulin, L.A.T.S. protector, a specific human thyroid stimulator, was shown to be present in the mother's serum. This stimulator may have been the cause of the neonatal hyperthyroidism.

Introduction

Rosenberg *et al.* (1963) first suggested that neonatal hyperthyroidism might be caused by transplacental passage of long-acting thyroid stimulator (L.A.T.S.). This immunoglobulin (IgG) is found in the serum of some patients with hyperthyroidism due to Graves's disease and has with few exceptions been present in the serum of both the mother and child in cases of neonatal hyperthyroidism. Thomson and Riley (1966), however, described a case in which L.A.T.S. was not detectable in the mother or child.

Adams and Kennedy (1967) noted the existence of another immunoglobulin in the serum of patients with Graves's disease, which they termed L.A.T.S. protector (L.A.T.S.P.), since it was able to compete with L.A.T.S. for binding to human thyroid fractions *in vitro* but did not stimulate mouse thyroid in the *in-vivo* McKenzie bioassay (Adams and Kennedy, 1971) or monkey thyroid (Knight and Adams, 1973). L.A.T.S.P. has been shown to stimulate human thyroid both *in vivo* (Adams *et al.*, 1974) and *in vitro* (Kendall-Taylor, 1973; Shishiba *et al.*, 1973), and since unlike L.A.T.S. it is present in most patients with Graves's disease (Stewart *et al.*, 1973) it has been suggested that it is likely to be a human thyroid stimulator responsible for the hyperthyroidism of Graves's disease.

We report a case of neonatal hyperthyroidism in which no L.A.T.S. could be detected in the mother or infant, but in which L.A.T.S.P. was present in the mother's serum.

Patients

The mother, aged 25 years, developed hyperthyroidism due to Graves's disease at the age of 12, when her basal metabolic rate was +45%. She was treated initially with potassium perchlorate and later with carbimazole. She relapsed on several occasions when antithyroid drugs were withdrawn and at age 20 underwent a partial thyroidectomy. Thyroid tissue showed changes compatible with hyperthyroidism, including focal lymphocytic infiltration with formation of germinal centres but no Askanazy cell change. She remained euthyroid thereafter. Her baby was born when she was 23 years of age. Eye signs of Graves's disease were present throughout, consisting of bilateral exophthalmos and left-sided lid retraction, but these did not necessitate treatment. At no time was pretibial myxoedema observed though slight splenic enlargement was noted before the thyroidectomy.

The child was born three weeks prematurely and weighed 2,180 g. At birth she was noted to be hyperkinetic, with a tachycardia of 140/min when resting, staring eyes, and a goitre. A thyroid function test at 3 days confirmed hyperthyroidism and she was treated with carbimazole 2.5 mg six-hourly and Lugol's iodine 1 drop eight-hourly from the tenth to 27th days with marked clinical improvement. Treatment was then withdrawn. Fourteen days later there was clinical and biochemical evidence of relapse of the hyperthyroidism and treatment was reinstated in decreasing doses till she was 9 months of age. Withdrawal was uneventful and she subsequently thrived.

Methods

Routine thyroid function tests included measurement of the serum protein-bound iodine (P.B.I.) on an AutoAnalyzer, residual thyroid hormone binding test (Thyopac-3; Amersham), and determination of the effective thyroxine ratio (E.T.R.; Mallinkrodt).

Thyroid-stimulating hormone (T.S.H.) was measured by radioimmunoassay using the method of Hall *et al.* (1971) with Medical Research Council H.T.S.H. 68/38 as standard. The thyrotrophin-releasing hormone test (T.R.H. test) was performed as described by Ormston *et al.* (1971). Thyroglobulin antibody levels were determined as described by Evered *et al.* (1973).

L.A.T.S. was measured using a modification of the McKenzie bioassay (Nutt *et al.*, 1974). L.A.T.S.P. was measured as described by Adams and Kennedy (1967) using a partially purified thyroid fraction of L.A.T.S. absorbing activity (L.A.A.). This was

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