be higher in the newborn than in the adult but was significantly so only at the last time interval.

Comparison of the disposition of digitoxin in newborn and adult rats is depicted in Fig. 3. Digitoxin disappeared at a similar slow rate from the plasma of both groups. However, the liver of the newborn contained a significantly lower amount of digitoxin than that of the adult at all time intervals. The lower amount of digitoxin in the liver of the newborn was due both to the smaller liver and to the lower concentration of glycoside in the liver. However, the liver of the newborn was able to concentrate the digoxin over that in plasma but not to the extent observed in the adult liver.

The disposition of digoxin in newborn and adult rabbits is shown in Fig. 4. The concentration of digoxin in the plasma or heart of the two groups was not significantly different. The amount of digoxin in the liver was lower in the newborn than the adult at all time intervals. This was mainly due to the difference in the ability of the livers of the two groups to concentrate digoxin.

Corresponding data obtained in the dog are shown in Fig. 5. Digoxin disappeared from the plasma at similar rates in the two groups. The amount of digoxin in the liver was significantly less in the newborn dogs at the 2- and 40-min time interval. Since the newborn dogs had relatively larger livers (4·1 per cent of body wt) than the adult dogs (3·3 per cent of body wt), the concentration difference was even greater. Both groups of dogs were able to concentrate digoxin in their livers but the adult could do this to a greater extent. No significant difference in the heart digoxin concentration was detected.

In conclusion, a decrease in the uptake of cardiac glycosides into the liver in newborn animals definitely is not limited to one cardiac glycoside (ouabain) or one species (rat). Although the largest difference was seen with ouabain in the newborn rat, differences existed with other glycosides and in other species as well. In newborn animals, the decreased rate of removal of cardiac glycosides probably results in a longer duration of their pharmacological action and may produce increased sensitivity to their toxic effects as well.

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REFERENCES

1. J. R. Fouts and R. H. Adamson, *Science, N.Y.* **129,** 897 (1959).
2. W. R. Jondorf, R. P. Maickel and B. B. Brodie, *Biochem. Pharmac.* **1,** 352 (1959).
3. C. D. Klaassen, *Toxic. appl. Pharmac.* **24,** 37 (1973).
4. E. Cox, G. Roxburgh and S. E. Wright, *J. Pharm. Pharmac.* **11,** 535 (1959).
5. H. J. Kupferberg and L. S. Schanker, *Am. J. Physiol.* **214,** 1048 (1968).
6. J. Q. Russell and C. D. Klaassen, *J. Pharmac. exp. Ther.* **183,** 513 (1972).
7. C. D. Klaassen, *J. Pharmac. exp. Ther.* **183,** 520 (1972).
8. C. D. Klaassen, in *The Liver, Quantitative Aspects of Structure and Function* (Eds. G. Paumgartner and R. Preisig), p. 402. S. Karger, Basel, Switzerland (1973).
9. J. Q. Russell and C. D. Klaassen, *J. Pharmac. exp. Ther.* **186,** 455 (1973).
10. M. C. Castle and G. L. Lage, *Res. Commun. Chem. Path. Pharmac.* **6,** 601 (1973).
11. R. G. D. Steel and J. H. Torrie, *Principles and Procedures of Statistics*, p. 67. McGraw-Hill, New York (1960).

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Transcortin levels in the blood of arthritic rats

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Cortisol is extensively used in the treatment of rheumatoid arthritis and is also effective in controlling the polyarthritis in the adjuvant rat [1]. Most of the endogenous cortisol in circulating blood is protein-bound to transcortin and albumin [2]. The binding between albumin and cortisol is weak (association constant 10^3–$10^5 M^{-1}$) and non-specific [2]. In contrast, transcortin binds cortisol firmly (association constant 10^7–$10^8 M^{-1}$) and is relatively specific [2]. Traditionally transcortin bound cortisol was thought to be inactive, but recent evidence suggests that it may be active in some tissues [3].

The inflammatory response in both rheumatoid arthritis [4] and adjuvant arthritis [5] affects protein biosynthesis *in vivo*. In this communication we have examined the effect of inflammation on the levels of transcortin in the blood of adjuvant arthritic rats.

Experimental. Arthritis was induced in Wistar strain male and female rats (150–200 g) by a method previously described [6]. Human strains (C, DT and PN) of tubercle bacilli were kindly supplied by the Ministry of Agriculture Veterinary Laboratories, Weybridge, Surrey. The adjuvant was introduced into the left hind foot-pad. The intensity of the induced inflammation was measured in both hind feet by immersing them separately to the hair line in a mercury bath connected to a pressure transducer linked to a Devices recorder. The initial measurements of foot volume were taken prior to injection of the adjuvant and subsequent measurements prior to collection of blood samples on the appropriate day.

Blood was drawn from the aorta of anaethetised animals and the serum was dialysed for 24 hr against saline at 4° to remove free cortisol. Serum transcortin levels were determined by the method of Milgrom [7]. In this method, 0·5 ml serum was incubated at 37° for 15 min with a large excess of [^{3}H]cortisol (1·9 μCi, 2·3 μg) to displace any endogenous cortisol remaining in the binding sites. Two ml. of a dextran coated charcoal suspension (0·25 g charcoal and 0·025 g dextran in 100 ml Tris–HCl buffer, pH 7·4) was added and the mixture shaken vigorously at 4° at a constant speed. At various intervals portions were removed, and after centrifuging to remove the charcoal, 0·2 ml of the supernatant was added to 5 ml of Bray's reagent [8] and the radio-

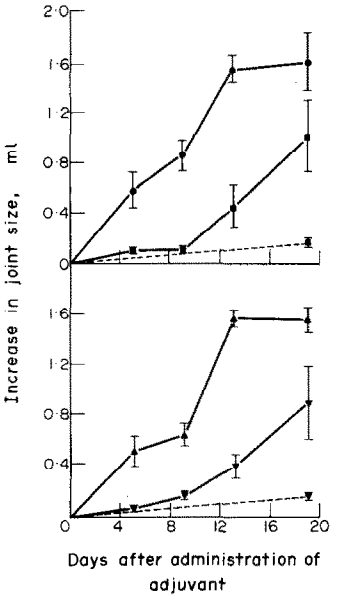

Fig. 1. Increase in size of the left (●——●) and right (■——■) hind feet of female rats, and left (▲——▲) and right (▼——▼) hind feet of male rats, after the injection of mycobacterial adjuvant into the left hind feet. Increases in the sizes of hind feet of non-injected control animals are represented by dotted lines. Each result represents the mean of five animals and vertical lines indicate S.E.M.

activity present determined in a liquid scintillation counting system (Beckman). In this way the transcortin-bound cortisol was determined and hence the amount of transcortin was estimated. Total protein levels were determined by the Biuret method [9].

Results and discussion. The initial inflammation at the site of injection subsided after 3–4 days as was found in our previous work [10] with this strain of rats and secondary inflammation started to develop from the 5th day after injection [10] (Fig. 1). Measurements of inflammation of both

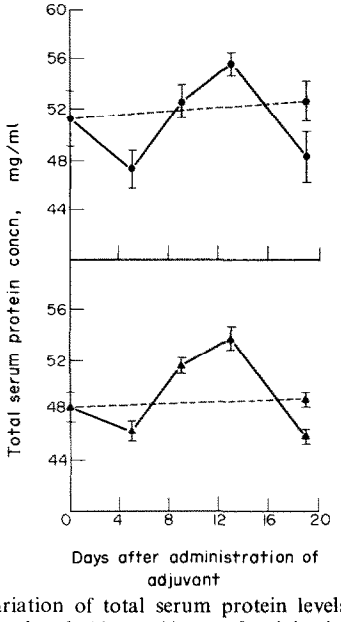

Fig. 2. Variation of total serum protein levels of female (●——●) and male (▲——▲) rats after injection of mycobacterial adjuvant. Control levels of non-injected rats are represented by dotted lines. Each result represents the mean of five animals and vertical lines indicate S.E.M.

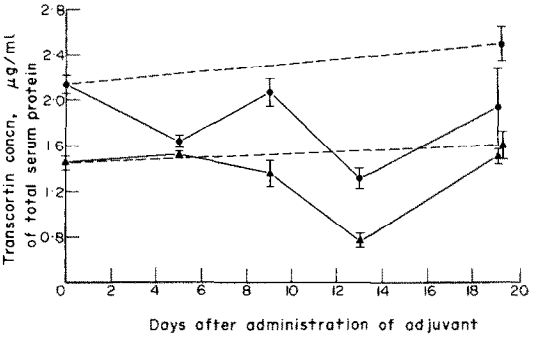

Fig. 3. Variation of transcortin levels of female (●——●) and male (▲——▲) rats after injection of mycobacterial adjuvant. Control levels of non-injected rats are represented by dotted lines. Each result represents the mean of five animals and vertical lines indicate S.E.M.

hind feet were taken from the 5th to the 19th day inclusive. No significant differences in severity were observed between male and female rats. Total protein levels (Fig. 2) fell slightly 5 days after the initial administration of adjuvant but rose sharply during the onset of secondary inflammation to reach maximum values, in excess of control values, 13 days after the initial injection. Again, no sex differences were observed. This rise may have reflected an increase in acute phase proteins which often accompany the onset inflammation since by the 19th day the levels had fallen to below normal. In contrast to the rise in total protein levels during the rapid onset of inflammation, the transcortin levels (Fig. 3) fell sharply in both male and female rats during this period. By the 19th day the transcortin levels had recovered slightly, but were still below normal. A sex difference in the transcortin levels of normal animals was observed, but this has previously been reported [11].

It is clear that during the phase when the onset of secondary inflammation occurred the transcortin levels in the blood fell despite a slight rise in the total amount of protein present. Whether this inverse relationship of transcortin levels in blood with the development of inflammation has any physiological significance is open to question. One possible consequence of a fall in transcortin levels could be a fall in total cortisol levels with an increased rate of cortisol metabolism [2]. Persellin has shown, however, that adjuvant arthritic rats develop hypertrophied adrenal glands and increased plasma corticosterone levels [12].

It is of interest that the transcortin and total cortisol levels rise during the latter stages of human pregnancy [2], a period when the symptoms of rheumatoid arthritis often regress [13]. However, a relationship between inflammatory disease and the levels of endogenous cortisol in 'free' and in 'bound' forms has still to be proven.

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REFERENCES

1. B. B. Newbould, *Br. J. Pharmac.* **21**, 685 (1963).
2. U. Westphal, in: *Biochemical Action of Hormones* (Ed. G. Litwack), Vol. 1, p. 209. Academic Press, New York (1970).

3. E. B. Thompson and M. E. Lippman, *Metabolism* **23,** 159 (1974).
4. J. P. Binette and K. Schmid, *Arth. and Rheumat.* **8,** 14 (1965).
5. J. S. Lowe and E. H. Turner, *Biochem. Pharmac.* **22,** 2069 (1973).
6. J. S. Lowe, *Biochem. Pharmac.* **13,** 633 (1964).
7. E. Milgrom and E. Baulieu, *Biochim. biophys. Acta*, **194,** 602 (1969).
8. G. A. Bray, *Analyt. Biochem.* **1,** 279 (1960).
9. A. G. Gornall, C. J. Bardawill and M. M. David, *J. biol. Chem.* **177,** 751 (1949).
10. A. J. Collins and D. A. Lewis, *Biochem. Pharmac.* **20,** 251 (1970).
11. R. R. Gala and U. Westphal, *Endocrinology* **77,** 841 (1965).
12. R. H. Persellin, G. W. Kittinger and J. W. Kendall, *Am. J. Physiol.* **222,** 1545 (1972).
13. P. S. Hench, E. C. Kendall, C. H. Slocumb and H. F. Polley, *Ann. rheum. Dis.* **8,** 97 (1949).

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Decreased rat brain acetylcholine utilization after heroin and cross tolerance to *l*-methadone*

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It is well known that morphine and related narcotic analgesics decrease acetylcholine (ACh) release from the peripheral and central nervous system [1–4]. In addition, brain ACh utilization and turnover are decreased [5, 6]. ACh utilization is an indirect measure of ACh turnover, since the compound used to inhibit its synthesis does not have an immediate effect. When the synthesis of ACh is reduced by hemicholinium-3 (HC-3) or acetylseco HC-3, drugs can be given at various times in order to determine if ACh utilization has been altered. A drug that alters brain ACh to a level below or above that found with an ACh depletor alone is interpreted to increase or decrease ACh utilization respectively. Narcotic analgesics, including *l*-methadone, antagonize brain ACh reduction produced by the ACh anti-synthesis agents like HC-3 and acetylseco HC-3 [5]. The present study summarizes results which indicate that acute administration of heroin causes a decrease in brain ACh utilization. Rapid partial cross tolerance to this action of heroin can be demonstrated to *l*-methadone given twice daily for only 4 days.

Male Holtzman rats, 20–24 days old (60–80 g), were housed 10–13/cage. They were on a 7:00 a.m. to 12:00 p.m. light and 12:00 p.m. to 7:00 a.m. dark cycle. They were given food and water *ad lib*. All drug doses were calculated as base. Two different treatments were used on various groups of animals. One group received 2·5 mg/kg of *l*-methadone i.p. for 2 days *b.i.d.*, 12 hr apart and then 5 mg/kg for the remaining 2 days on the same dose schedule. The other group of rats was given 0·9% NaCl in a volume equivalent to those receiving *l*-methadone *b.i.d.* for 4 days. This dose schedule of *l*-methadone allowed the young rats to gain weight at the same rate as the 0·9% NaCl controls. About 5–10 per cent of the animals given methadone died. On day 5, the rats were given simultaneously heroin i.p. and 5 μg acetylseco HC-3 (0·25 μg/μl) intraventricularly (ivt.) under diethyl ether anesthesia 30 min prior to being guillotined. Acetylseco HC-3 is especially useful because it is a choline acetyltransferase inhibitor that causes significant decreases in brain ACh compatible with survival [7].

The brain minus the cerebellum was rapidly removed, weighed and homogenized in acetonitrile to which 25 nmoles proprionylcholine (PCh) iodide had been added as internal standard. The samples were prepared for pyrolysis gas chromatography (GC) by the method of Szilagyi *et al.* [8]. However, the GC conditions were slightly altered for convenience. Stainless steel columns, 8 ft in length, packed with Chromasorb W (HMDS) coated with 20% Carbowax 6000, were used. Column temperature was 145°, flame ioni-

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Table 1. Effects of heroin on brain acetylcholine utilization in rats treated with 0·9% NaCl or *l*-methadone

Treatment	N	0·9% NaCl Mean ± S.E. ACh (nmoles/g)	N	*l*-Methadone Mean ± S.E. ACh (nmoles/g)	P value*
Controls	11	23·9 ± 0·7			
Acetylseco HC-3 (5 μg)	9	11·1 ± 0·5			<0·001
Acetylseco HC-3 (5 μg) + heroin (mg/kg)					
0·1	8	12·9 ± 1·1			
0·32	9	15·6 ± 1·0	9	12·3 ± 0·8	<0·01
0·56	10	17·9 ± 0·8			
1·0	9	23·8 ± 1·5	9	14·7 ± 0·9	<0·001
3·2	9	17·1 ± 1·2	9	18·4 ± 1·1	NS
10·0			13	15·1 ± 0·4	

* Group comparison "t" in which the effects of acetylseco HC-3 alone were compared to controls, or the 0·9% NaCl- and *l*-methadone-treated groups were compared. Heroin plus acetylseco HC-3 was given to rats treated 4 days with 0·9% NaCl or *l*-methadone. NS = not significant.