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DISAPPEARANCE OF [1α-^{3}H]-CHLORMADINONE ACETATE IN THE PLASMA AND RED CELLS OF RHESUS MONKEY

VIMLA LAUMAS, A. FAROOQ and K. R. LAUMAS
Department of Reproductive Biology, All India Institute of Medical Sciences, New Delhi, 110016, India

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SUMMARY

The disappearance of chlormadinone acetate (CAP) in plasma and red cells of monkeys after i.v. injection of [1α-^{3}H]-CAP is presented. The plasma disappearance curve shows a biexponential function and is analysed on the basis of a two-compartment model. CAP showed an initial rapid disappearance (half-life of 68 min) followed by a slow disappearance (half-life of 35.1 h). The half-life of CAP was much longer compared with the half-lives of oestradiol and progesterone. The disappearance curve followed a similar pattern of prolonged half-life in red cells also. The metabolic clearance rate of CAP was found to be 102.6 l./day. Although the half-life of CAP in the monkey is shorter than in women the MCR is almost in the same range (126.6 l./day)—indicating a slow clearance of the steroid from the body. The studies showed that although qualitatively the pattern of metabolism of CAP in monkey and women was similar, there were quantitative differences.

INTRODUCTION

There is lack of knowledge on the metabolism and turnover of progestational steroids in man and other primates. Monkey is preferred as an experimental animal because toxicity studies for progestational compounds are usually carried out on three different species of animals, of which the monkey is usually one. This paper describes the metabolic clearance rate of [1α-^{3}H]-chlormadinone acetate (CAP) in the plasma and red cells of female monkeys. In particular, the plasma disappearance curve has been analysed mathematically to calculate the metabolic clearance rate (MCR) of the steroid.

MATERIALS AND METHODS

Animals. Adult female rhesus monkeys were purchased from T. E. Patterson & Co., New Delhi. These were housed in air-conditioned quarters and fed on pellet diet manufactured by Hindustan Lever, Bombay. The animals were given water *ad libitum* and were followed for their menstrual cycle by taking daily vaginal smears. Animals which were regularly cycling and had an average menstrual cycle of 28 ± 3 days were chosen for the study.

Injection solution. 55.0 μCi [1α-^{3}H]-Chlormadinone acetate (222 μCi/mg) was dissolved in 5.0 ml 10% ethanolic saline and injected intravenously in the leg vein of each monkey.

Blood collection. After injection, blood samples were collected from the opposite leg vein at intervals of 10 min, 30 min, 1, 4, 8, 24, 48, 96 and 120 h in heparinized tubes. Plasma was separated from the red cells by centrifugation.

Extraction of steroids. After centrifugation of the blood samples, the radioactivity due to [1α-^{3}H]-CAP was extracted separately from plasma and red cells as per the method of Pearlman *et al.* [1].

The ether extract remaining after taking an aliquot for counting was dried under nitrogen and spotted on a 0.25 mm silica gel thin layer plate, along with pure chlormadinone acetate standard (E. Merck, Darmstadt, W. Germany). The solvent system used was benzene:ether (1:2, v/v). The spot due to authentic CAP was located under ultraviolet light. The area corresponding to the standard and 1 cm above and below it was scraped out and thrice extracted for radioactivity due to chlormadinone acetate with chloroform:methanol (1:1, v/v). The extracts were pooled. Out of the pooled extract, an aliquot was taken for estimation of radioactivity and the rest was dissolved in 2 ml acetone for crystallization to constant S.A. [2].

RESULTS

Plasma disappearance of [1α-^{3}*H*]-*CAP and its metabolites*

The disappearance of CAP was studied in the plasma of rhesus monkeys, at different time intervals after single intravenous injections of 55.0 μCi of [1α-^{3}H]-CAP. The radioactivity was analysed into total, free and conjugated radioactivity and radioactivity present specifically as CAP. The values are plotted as logarithm of percent injected dose/l. plasma against the time intervals. The data for the plasma disappearance of CAP for four rhesus monkeys No. 7, 8, 9 10 are presented in Figs. 1, 2, 3 and 4.

Figure 1, shows that in monkey No. 7 radioactivity due to CAP and its metabolites disappears initially rapidly upto a period of 4 h followed by a slow disappearance upto 120 h when the experiment was terminated. The free radioactivity followed a disappearance pattern identical to that of total radioactivity except that the levels were lower at different time intervals. The radioactivity specifically analysed as CAP

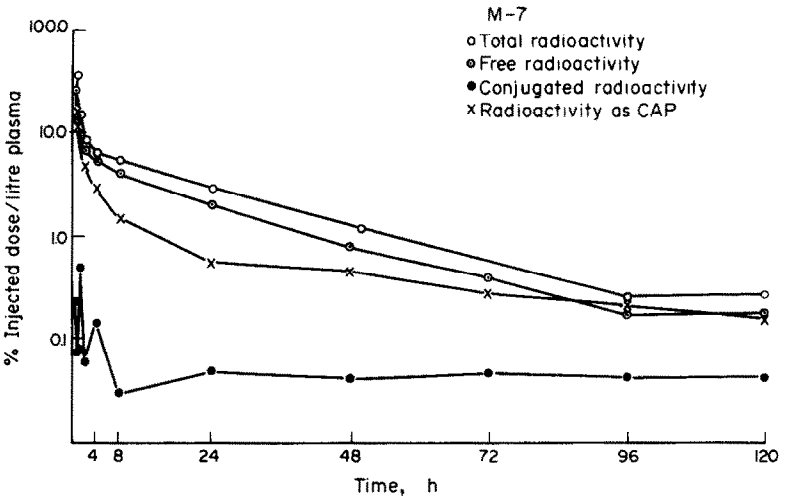

Fig. 1. Disappearance of radioactivity in the plasma of monkey No. 7 after an intravenous injection of 55.0 μCi of [1α-^{3}H]-chlormadinone acetate.

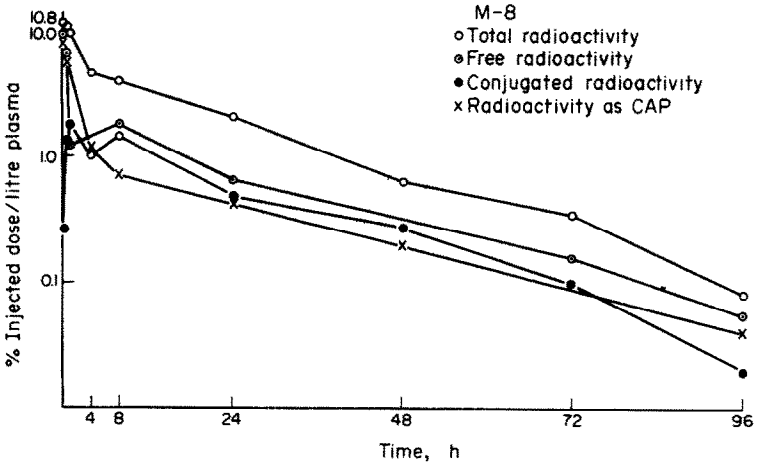

Fig. 2. Disappearance of radioactivity in the plasma of monkey No. 8 after an intravenous injection of 55.0 μCi of [1α-^{3}H]-chlormadinone acetate.

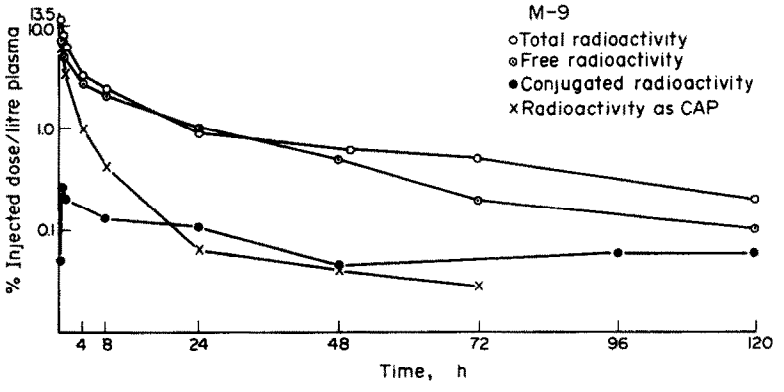

Fig. 3. Disappearance of radioactivity in the plasma of monkey No. 9 after an intravenous injection of 55.0 μCi of [1α-^{3}H]-chlormadinone acetate.

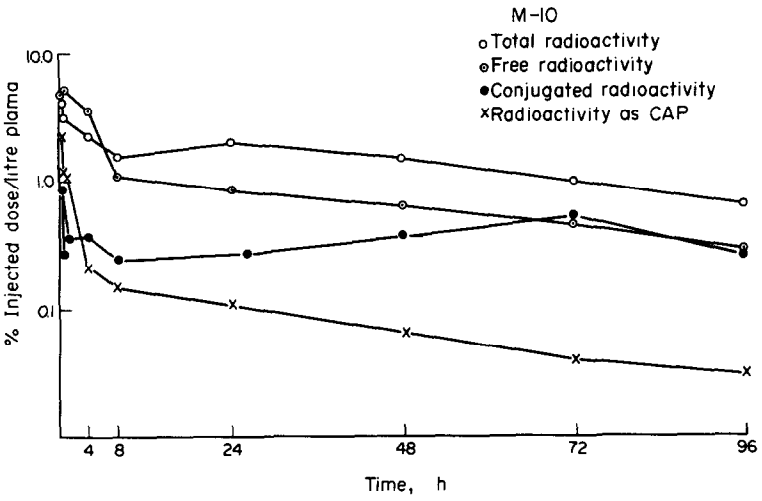

Fig. 4. Disappearance of radioactivity in the plasma of monkey No. 10 after an intravenous injection of 55.0 μCi of [1α-^{3}H]-chlormadinone acetate.

showed an initial rapid fall followed by a somewhat gradual decline and a subsequent slow disappearance as in case of total and free radioactivity. The levels of CAP at 120 h were of the order of 0.15 percent of the injected dose per litre plasma. The radioactivity representing conjugated metabolites of CAP showed some fluctutations in its plasma levels initially. However, by 24 h a level of the order of 0.05 percent of the injected dose per litre plasma was attained, which remained constant at the later time intervals.

The general pattern of disappearance of [^{3}H]-CAP in the plasma of monkey No. 8, 9, 10 as total, free and conjugated radioactivity and the radioactivity specifically present as CAP is similar to that seen in monkey No. 7. The steroids were analysed upto 96 h in monkey No. 8 and 10 while the analysis was carried out upto 120 h in case of monkey No. 9.

Plasma disappearance of [1α-^{3}H]-CAP

The values for the radioactivity present specifically as CAP and fully corrected for recovery in plasma taken at different time intervals after the intravenous injection of [^{3}H]-CAP to the four monkeys are shown in Figs. 5, 6, 7 and 8. These are plotted as logarithm of percent injected dose per litre plasma against the time. The disappearance curves have been drawn as the best fit through the actual values observed at different time intervals. In all four cases there was an initial rapid drop in the plasma radioactivity to about 2–24 percent, of the injected dose per litre plasma. All the four curves show an initial drop upto about 4 h with an average half life of the order of 63 min. From 8 h upto 120 h there was a very slow decline in plasma concentration of CAP with an average half life of 35.1 h (Table 1).

Analysis of the disappearance curve

The disappearance curves, in general, showed two distinct rates of disappearance, an initial rapid followed by a slow disappearance. In some cases, the disappearance curve appeared to show three rates of disappearance. However, for the sake of uniformity all the curves have been analysed using biexponential equations for reasons explained under "Discussion". The concentration of radioactivity in the plasma due to CAP as a function of time could be deduced from the characteristics of the disappearance curves and expressed as $X = Ae^{-\alpha t} + Be^{-\beta t}$ where X = injected dose per litre of plasma as CAP at time t. B is the intercept on the ordinate of the extrapolated part of the curve. $A + B$ is the corresponding intercept from the earlier part of the curve. β has been calculated

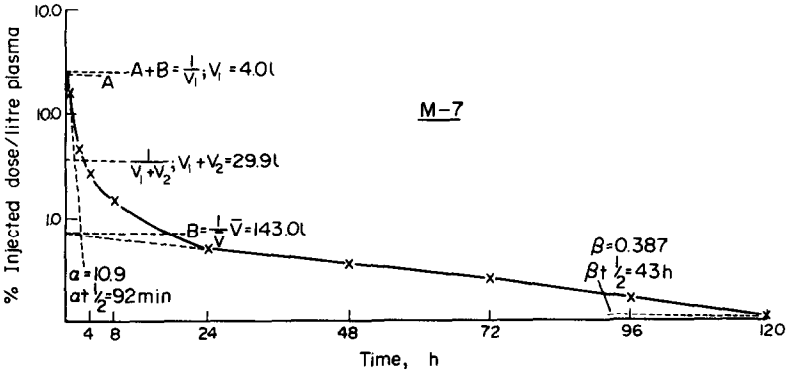

Fig. 5. Disappearance of radioactivity specifically analysed as chlormadinone acetate in the plasma of monkey No. 7 after an intravenous injection of 55.0 μCi of [1α-^{3}H]-chlormadinone acetate.

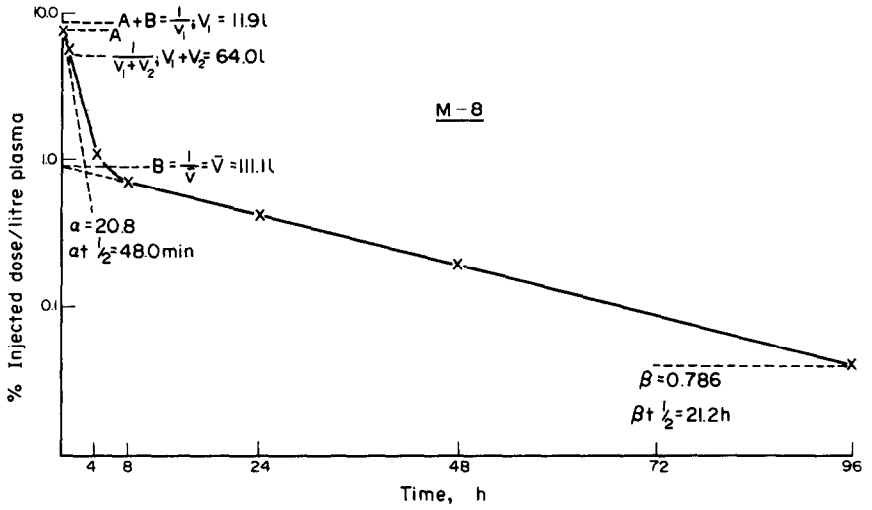

Fig. 6. Disappearance of radioactivity specifically analysed as chlormadinone acetate in the plasma of monkey No. 8 after an intravenous injection of 55.0 μCi of [1α-^{3}H]-chlormadinone acetate.

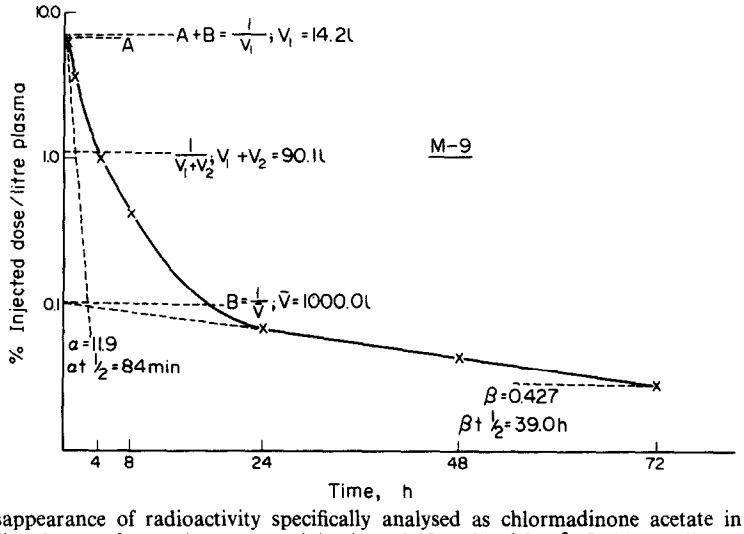

Fig. 7. Disappearance of radioactivity specifically analysed as chlormadinone acetate in the plasma of monkey No. 9 after an intravenous injection of 55.0 μCi of [1α-^{3}H]-chlormadinone acetate.

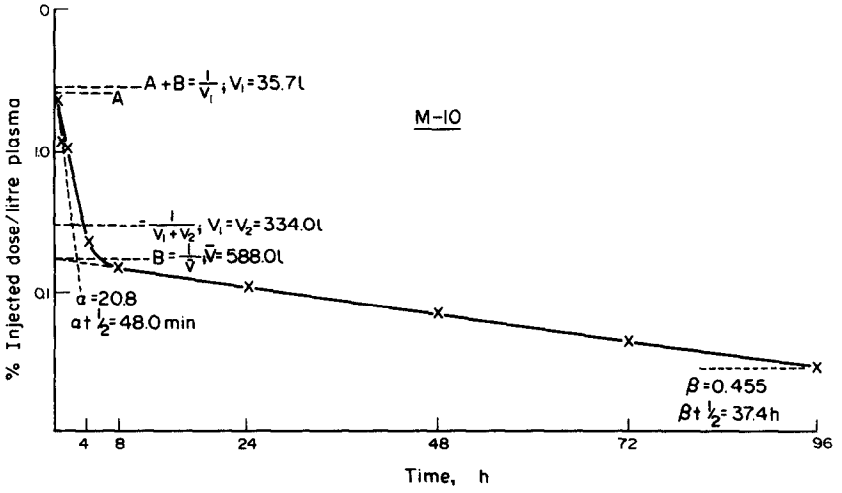

Fig. 8. Disappearance of radioactivity specifically analysed as chlormadinone acetate in the plasma of monkey No. 10 after an intravenous injection of 55.0 μCi of [1α-^{3}H]-chlormadinone acetate.

Table 1. Calculated values of A, B, α, β, $\alpha t^{1/2}$, $\beta t^{1/2}$ and metabolic clearance rates of chlormadinone acetate in monkeys

	A % dose/l.plasma	B % dose/l.plasma	α days^{-1}	β days^{-1}	$\alpha t^{1/2}$	$\beta t^{1/2}$	MCR l./day
M-7	24.3	0.7	10.9	0.39	92 min	43.0 h	24.8
M-8	7.6	0.9	20.8	0.79	48 min	21.2 h	66.2
M-9	6.9	0.1	11.9	0.43	84 min	39.0 h	122.8
M-10	2.6	0.17	20.8	0.44	48 min	37.4 h	196.7
Mean			16.1	0.51	68 min	35.1 h	102.6

from the equation $\beta = 1440\ (\ln^2/t^{1/2})$ where $t^{1/2}$ is in minutes β is days^{-1} and β (days^{-1}) = approx. $1000/t^{1/2}$. Both B and β have been calculated from the values at 8, 24, 48, 72, 96 and 120 h for monkey No. 7. The B and β values in case of monkey No. 8 and 10 have been calculated from values between 8 to 19 h. The B and β values in case of Monkey No. 9 have been calculated from the values between 8 to 36 h. α is calculated as follows: The later part of the curve is extrapolated intercepting the ordinate at B and the resulting calculated plasma concentrations subtracted from the measured values. These corrected concentrations from 0–4 h are plotted semi-logarithmically and the slope is α.

Table 1 gives the value for A, B, α, β, $\alpha t^{1/2}$, $\beta t^{1/2}$ and the MCR which has been calculated by the following equation:

$$MCR = \frac{100}{A/\alpha + B/\beta} = \frac{100\,\alpha B}{A\beta + B\alpha}.$$

Disappearance of [1α-^{3}H]-CAP and its metabolites in the red cells

The erythrocytes were obtained from the blood samples of monkey No. 9 collected at different time intervals and extracted for total, free and conjugated radioactivity by a procedure similar to that used for plasma samples. The free radioactivity from the red cells was further chromatographed to determine the amount of CAP as such. Figure 9 gives the pattern of disappearance of total, free and conjugated radioactivity and also radioactivity present as CAP in the red cells of monkey No. 9. The levels of radioactivity present in samples collected at 96 and 120 h were very low and hence not included. All the results are expressed as percent injected dose in red cells/litre blood.

The total and free radioactivity due to CAP and its metabolites in the red cells showed an initial rapid disappearance followed by a slow disappearance as seen in plasma too. However, the levels of the radioactivity at different time intervals were much lower than in plasma. The pattern of disappearance of radioactivity specifically analysed as CAP was also similar to that seen in plasma in general. It showed an initial very rapid drop followed by somewhat slower disappearance upto 24 h. Between 24 h and 72 h it declined very slowly.

DISCUSSION

An interesting feature of the results presented in this paper was the long half-life and low MCR of chlormadinone acetate in the monkey. The disappearance of total radioactivity and the radioactivity specifically analysed as chlormadinone acetate showed a similar pattern of initial rapid disappearance followed by a slow disappearance. The average half-life for the first rapid disappearance part was 68 min and for the slow disappearance period was 35.1 h. The disappearance curve showed a biexponential function, but in some instances there was evidence

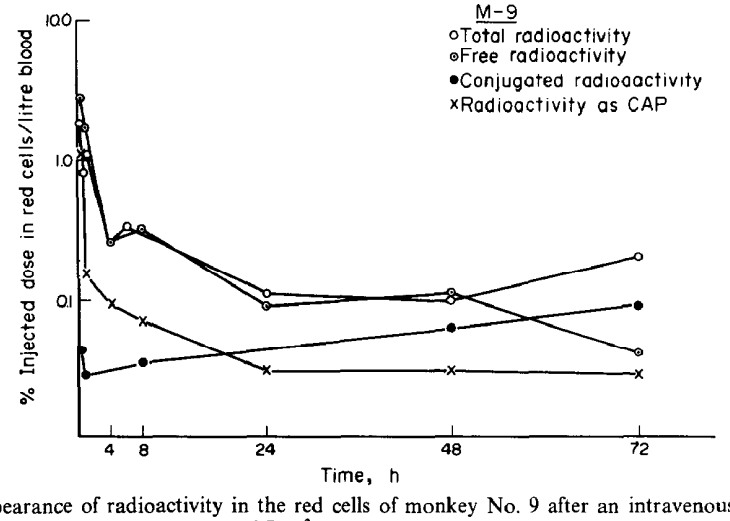

Fig. 9. Disappearance of radioactivity in the red cells of monkey No. 9 after an intravenous injection of 55.0 μCi of [1α-^{3}H]-chlormadinone acetate.

for three types of disappearance. Since Sharney, Wasserman and Cevirtz [3] have shown that three or more compartmental systems could be described by the two compartment model, all disappearance curves were analysed as a two compartment model. The metabolism of natural sex hormones, progesterone and oestradiol, has also been described using a two compartment model [4, 5].

Chlormadinone acetate is different from natural sex steroids in possessing a long half-life, and a very slow disappearance from the plasma of women [7]. Other contraceptive steroids have also shown similar characteristics. Norgestrel showed an initial half-life of 38.8 min and a second half-life of 45.8 h in women [6] and CAP showed an initial half-life of 2.40 h followed by a slow disappearance of 80.1 h in women [7]. The initial and later half-lives of CAP in monkey are somewhat shorter as compared with that in women although the general pattern of disappearance was similar. This difference may be due to lower body mass and species difference between man and monkey.

MCR of CAP in the four monkeys studied was 24.8, 66.2, 122.8 and 196.7 l./day. Considerable variation was found in the *MCR* value of different monkeys. Considerable variation was also found in the *MCR* value of testosterone and mestranol in human subjects [8, 9]. The average *MCR* of CAP in monkeys is 102.6 l./day, which is quite low compared with the *MCR* of natural sex steroids in women. Progesterone has an *MCR* of 2,443 l./day [4], while oestrone and oestradiol have 2,210 and 1,350 l./day respectively [5]. When *MCR* of CAP was calculated in women [7], it was 126.6 l./day which is still quite low compared with the *MCR* of oestradiol and progesterone. The *MCR* of CAP in monkeys in the present study was comparable to that in women.

The slow disappearance of CAP and its low metabolic clearance could be attributed to a number of factors. An important factor could be storage of the steroid in the body fat. A high uptake of radioactivity in the fat of rats and women [11] was observed after an intravenous injection of 3H-CAP. In women this radioactivity on analysis was found to be specifically due to CAP. The other possibilities could be its binding to plasma proteins and storage in the red cells. In the present study small amounts of CAP could be found in the red cells and it had a long half-life. Enterohepatic circulation of CAP could also contribute to its low MCR value. A considerable faecal excretion of CAP, megestrol acetate and their metabolites has been reported [12] and it could be attributed to the enterohepatic circulation of these steroids.

In the red cells, total radioactivity representing CAP and its metabolites and radioactivity analysed specifically as CAP showed an initial rapid disappearance followed by a slow disappearance resembling the pattern in the plasma. However, levels of the steroids were much lower than that seen in the plasma thereby indicating greater affinity of CAP for plasma than for red cells. The red cells are known to possess active enzymes which are able to convert oestrone to oestradiol [13, 14]. The uptake and long half-life of CAP in the red cells may have a role in the transport and action of CAP which needs further elucidation.

The analysis of total radioactivity in the plasma revealed the presence of considerable amounts of free and conjugated metabolites. The structure of the metabolites of chlormadinone acetate has not yet been determined and no information is available as to their biological activity. It is therefore, not possible to ascribe any significance to the levels of the free and conjugated metabolites of CAP. The study revealed that there are similarities in the metabolism of CAP in monkey and women. Monkey may thus provide a useful model for the study of metabolism of contraceptive steroids.

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