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INTERACTIONS OF STEROIDS WITH SERUM LIPOPROTEINS

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Abstract—The finding that testosterone undecanoate (a non-polar steroid) after oral administration to rats was completely bound to the lipoproteins and chylomicrons in the blood, motivated us to investigate the interaction of various steroids with plasma lipoproteins. Very low density and low density lipoproteins were isolated from pig serum by polyanion precipitation. The binding of steroids to the isolated lipoproteins was demonstrated by ultracentrifugation, equilibrium dialysis and electrophoresis and a positive correlation was found between the lipophilic character of a substance and its degree of binding to the lipoproteins. A lipoprotein solution was shown to be an excellent solvent for lipophilic substances and in particular could be used for intravenous administration of such substances. The biological activity of testosterone undecanoate, when administered intravenously in such solutions, was retained.

The binding of non-polar compounds to serum lipoproteins was suggested by Avigan [1], who showed that some sterols dissolve in lipoprotein solutions. A similar binding of non-polar compounds to serum lipoproteins was also suggested by Rudman *et al.* [2], who found that when compounds such as testosterone were added to lipaemic sera, a part of the added compound was bound to the lipoproteins.

Roberts and Szego [3] demonstrated that oestriol in human serum was mainly bound to the β-lipoprotein fraction, but the results of this investigation were disputed by Bischoff and Pilhorn [4]. The latter authors determined the solubilities of oestriol in albumin, α-globulin and lecithin solutions and found that they were of the same order of magnitude as the solubility of oestriol in serum. They therefore concluded that oestriol in serum was not bound by the lipoproteins. In our laboratory it was found that non-polar compounds (testosterone undecanoate and 5α-dihydrotestosterone undecanoate) after oral administration to rats were bound to the lipoprotein fraction of the blood (to be published). This finding motivated us to investigate the binding of steroids and some other lipophilic substances to lipoproteins *in vitro*.

MATERIALS AND METHODS

Chemicals. The chemicals used were of A.R. quality unless otherwise indicated. The following labelled substances were used: [4-^{14}C]testosterone from New England Nuclear Corp., Boston, MA, U.S.A., radiochemical purity approximately 97 per cent, [4-^{14}C]nandrolone from Radiochemical Centre, Amersham, England, radiochemical purity approximately 98 per cent, and [1,2,^{3}H]testosterone undecanoate, labelled by Radiochemical Centre, radiochemical purity >99 per cent.

Biological materials. Male Wistar rats of the Orga-TNO strain, body wt 180–200 g, obtained from TNO*, were used in all experiments. Pig serum was prepared by centrifuging fresh pig blood obtained from a slaughterhouse. Human serum was prepared by centrifuging fresh blood from an adult male.

Thin layer chromatography (*t.l.c.*). T.l.c. was performed in closed non-equilibrated tanks on silica gel plates (Silica Gel HF 254 + 366, Merck, Darmstadt, West Germany). The following systems were used: for testosterone and nandrolone determination, toluene–ethyl acetate (1:1), for testosterone undecanoate determination, *n*-hexane–chloroform–ethyl acetate (4:5:1) and for simultaneous determination of testosterone and testosterone undecanoate, *n*-hexane–chloroform–ethyl acetate (4:5:2). Reversed phase chromatography was performed on silica gel plates impregnated with liquid paraffin by the immersion technique in non-equilibrated closed tanks. Systems: (1) acetone–water (7:3), (2) methanol–acetone (9:1).

Fluctuations in the R_f values were eliminated with standards according to the procedure of Galanos and Kapoulas [5].

Chemical determinations. Determination of protein content was carried out either by the biuret reaction or by the method described by Lowry *et al.* [6]. Determinations of cholesterol and triglicerides were carried out automatically according to Zlatkis *et al.* [7] for cholesterol and according to Noble *et al.* [8] for triglycerides. The extraction procedure for both was according to Cramp *et al.* [9]. Steroids were determined after saturation of the solution with sodium chloride, and extraction with ethyl acetate. The extracts were evaporated at 60° under a stream of nitrogen. The residue was subjected to t.l.c. and after

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development the spots were scraped off and extracted with methanol or acetone. The steroid concentration was determined colorimetrically with isonicotinic acid hydrazide or by gas–liquid chromatography with a Hewlett Packard gas chromatograph model F & M 402.

Isolation and characterization of lipoproteins. Human serum very low density lipoproteins (VLDL) together with low density lipoproteins (LDL) were isolated according to Burstein *et al.* [10]. For the isolation of pig serum VLDL + LDL the method of Burstein was modified: to 2 l. of serum 200 ml of a 5% heparin-sodium solution (U.S.P. quality) and 250 ml of a 1 M $MnCl_2$ solution were added; a precipitate appeared at once. The mixture was centrifuged for 15 min at 4500 *g*, the supernatant was decanted and the precipitate was dissolved in 30 ml of a 10% $NaHCO_3$ solution. The manganese, associated with the lipoproteins, precipitated as the bicarbonate salt and was removed by centrifugation. The precipitate was washed twice with 10 ml of 10% $NaHCO_3$ solution. The supernatants were combined and mixed with 2 l. of Tris–HCl buffer (0·02 M, pH 7·7) and the lipoproteins were completely precipitated by addition of 62 ml of 2 M $MgCl_2$ solution. The precipitate was separated by centrifugation (15 min at 5000 *g*) and dissolved in 60 ml of 5% NaCl solution. Another precipitation was performed as described above for further purification of the lipoproteins. The precipitate was then dissolved in 16 ml of 10% sodium citrate solution. This solution was dialyzed against Tris–HCl–NaCl buffer (0·02 M Tris, 1% NaCl with HCl to pH 7·7) for 24 hr at 4°. In order to remove the heparin-sodium, the solution was dialyzed against 5% $BaCl_2$ solution for 24 hr at 4°. The insoluble heparin–barium complex was then removed by centrifugation. The supernatant was dialyzed again for 24 hr against Tris–NaCl–HCl buffer at 4°. The final (colloidal) solution had a milky appearance. After isolation the lipoprotein solution was stored at −20°. Before use the solution was diluted with distilled water, usually in the ratio 1:20. For convenience this lipoprotein solution is abbreviated to LPS; it was weakly opalescent. The isolated lipoprotein solution contained approximately 20 mg protein, 30 mg cholesterol and 17 mg triglycerides per ml. The isolated lipoproteins had different electrophoretic mobilities than the lipoproteins in serum. The lipoprotein solution can be protected against degradation (peroxide formation [11, 12]) by adding disodium edetate.

Solubilization of steroids in LPS and serum. Steroids were dissolved in acetone. The concentration of this solution depends on the desired final concentration in LPS or serum. Twenty-five microlitres of this solution was injected forcefully into 1 ml of LPS or serum with the aid of a Hamilton syringe. This step is a critical one, since when the solution in acetone is injected slowly the distribution of the steroid in LPS or serum is irregular. The complex formed between steroid and LPS is not a true solution but for the purpose of this publication will be deemed to be such.

Determination of relative solubilities of substances in LPS. Solutions of substances in LPS were prepared, as described above, in four concentrations, viz. 0·25, 1·25, 2·5 and 5 mM. The time between preparing a solution and precipitation of dissolved substances was taken as a criterion of the degree of solubility.

Electrophoresis. Electrophoresis was performed on agar–agarose gel according to Noble [13] as modified by Dyerberg *et al.* [14], though in our experiments no albumin was added to the buffer. Agar was purchased from Difco (Batch no. 0140) and agarose (Indubiose A 37) was purchased from l'Industrie Biologique Française S.A., Gennevilliers, France. Lipid staining was performed according to Dyerberg *et al.* [14].

Ultracentrifuge experiments. An IEC International Preparative Ultracentrifuge model B 60 with rotor 488 was used. LPS with or without steroid of density 1·01 (prepared by adding sucrose) was centrifuged at 80,000 *g* for 16 hr at 10°. The separated supernatant fraction, infranatant fraction and residue were analyzed for their protein, cholesterol, triglyceride and steroid content. Rat plasma without chylomicrons (removed by centrifugation at 25,000 *g* for 20 min) was mixed with potassium bromide solution (final density 1·21) and then centrifuged for 20 hr at 100,000 *g* at 4°. After the run the supernatant which contained the lipoproteins was separated from the rest of the plasma protein.

Determination of radioactivity. A Packard Liquid Scintillation Spectrometer model 3375 was used. Instagel (Packard) was used as scintillation liquid.

Autoradiography. Autoradiography of small amounts of [^{3}H]testosterone undecanoate on electropherograms or on silica gel layers was carried out by spraying the plates with Omnispray® (New England Nuclear) and storing them with Kodak Royal Blue® film at −80° for several days.

Scanning of electropherograms. The electropherograms were scanned with a gasflow proportional counter (type LB 2721, Berthold, Wildbad, West Germany).

Equilibrium dialysis. The experiments were carried out at 25° for 18 hr. The steroids dissolved in acetone, were added either to LPS inside the bag (1·25 μg/5 ml) or to the buffer outside the bag (1·25 μg/10 ml). After dialysis the radioactivity of samples from inside and outside and of the membrane was determined. Binding was calculated with the formula of Slaunwhite [15]:

$$\text{Percentage binding} = 100 \times \left(1 - \frac{\text{dis/min outside} \times \text{volume inside}}{\text{dis/min inside} \times \text{volume outside}}\right)$$

Solubilization of solid testosterone undecanoate in lipoprotein solutions. Aliquots of 50 μl of a [^{3}H]testosterone undecanoate solution in acetone (concentration 5 mg/ml) were placed on small pieces of filter paper, Whatman 3 mm (all pieces had the same surface area), and the acetone was evaporated. The pieces were shaken at 25° with 2·5 ml of lipoprotein solutions of

Table 1. Ranking of substances in increasing solubility in LPS and corresponding lipophilic character, together with R_f values in system 1 = acetone–water (7:3) and system 2 = methanol–acetone (9:1)

Substances*	Solubility	Lipophilic character	R_f values System 1	System 2
Dexamethasone	1	1	0·95	0·88
Dexamethasone-21-acetate	2	2	0·93	0·89
Testosterone	3	5	0·85	0·85
Testosterone propionate	4	9	0·73	0·88
16α-Hydroxy nandrolone	5	4	0·89	0·83
Mestranol	6	8	0·76	0·84
Progesterone	7	10	0·73	0·86
16α-Methyl progesterone	8	11	0·68	0·86
Nandrolone acetate	9	7	0·78	0·87
Nandrolone	10	3	0·90	0·86
16α-Ethyl progesterone	11	13	0·63	0·86
Nandrolone propionate	12	12	0·64	0·87
Lynestrenol	13	14	0·61	0·86
Nandrolone phenylpropionate	14	16	0·52	0·87
Lynestrenol acetate	15	21	0·38	0·73
Dexamethasone-21-phenylpropionate	16	6	0·84	0·91
Dexamethasone-21-palmitate	17	20	0·39	0·86
Nandrolone-3′3′-diphenylpropionate	18	17	0·46	0·87
Nandrolone-2′2′-diphenylpropionate	19	18	0·42	0·86
Testosterone phenylpropionate	20	15	0·60	0·86
Testosterone undecanoate	21	24	0·19	0·73
Lynestrenol phenylpropionate	22	23	0·23	0·64
Nandrolone hexanedioat	23	19	0·42	0·88
Nandrolone undecanoate	24	22	0·20	0·88
Nandrolone palmitate	25	27	0·04	0·59
Retinol palmitate	26	28	0·04	0·20
α-Tocopherol acetate	27	26	0·07	0·48
Nandrolone laurate	28	25	0·09	0·75
α-Tocopherol	29	29	0·04	0·18

Correlation coefficient of the two rankings is 0·92.

* Dexamethasone = 9α-fluoro-11β,17,21-trihydroxy-16α-methyl-$\Delta^{1,4}$-pregnadiene-3,20-dione. Testosterone = 17β-hydroxy-Δ^4-androstene-3-one. Nandrolone = 17β-hydroxy-Δ^4-estrene-3-one. Mestranol = 17α-ethinyl-$\Delta^{1,3,5,(10)}$ estratriene-3,17β-diol-3-methylether. Progesterone = Δ^4-pregnene-3,20-dione. Lynestrenol = 17α-ethinyl-Δ^4-estrene-17β-ol.

various concentrations. After 1, 2, 4 and 6 hr the solutions were centrifuged for 10 min at 2000 *g* and the radioactivity of the supernatant was measured.

MLA-test (Hershberger test) [16]. Biological activity of testosterone undecanoate in LPS was determined in the MLA-test. In this test the growth of the seminal vesicles, ventral prostate and levator ani muscle of young male castrated rats (approx. 50 g) was determined by weighing.

RESULTS

Solubilization of substances in LPS

Influence of the medium. When solutions of steroids in LPS were prepared it was apparent that increasing ionic strength decreased the solubility of the steroids. For this reason solutions of steroids in LPS were made isotonic with glucose instead of sodium chloride.

Influence of the lipophilic character of a substance on its dissolution in LPS. The solubilities of all the substances mentioned in Table 1 were determined in the same experiment. For some substances some of the higher concentrations could not be achieved because their solubility in acetone was too low. Exact values could not be determined; only relative solubilities could be given. Extreme examples are dexamethasone which crystallizes 2 hr after preparation (concn 0·25 mM; highest possible concentration) and α-tocopherol that remained in solution up to 24 hr after preparation (concn 1·25 mM). In Table 1, column 2, the substances are ranked according to their solubility in LPS. The lipophilic character of these substances was determined by reversed phase chromatography. In this procedure the lipophilic character of a substance is correlated with its R_f value. In Table 1, column 3, the substances are ranked according to their lipophilic character and in columns 4 and 5 the R_f values of the substances in systems 1 and 2 are given. The correlation coefficient between the two rankings calculated according to Spearman as described by Pearson *et al.* [17] is 0·92. This is significant at $P < 0{\cdot}001$.

A solution of human serum VLDL + LDL, diluted 1:20, with distilled water showed qualitatively the

Table 2. Ultracentrifugation of steroid solutions; values are in percentage of recovered quantity

	Residue	Infranatant	Supernatant*
In buffer			
Nandrolone	0	100	—
Testosterone	84	16	—
Testosterone undecanoate	99	1	—
In LPS			
Nandrolone	4	85	11
	3	84	13
Testosterone	17	72	11
	14	74	12
Testosterone undecanoate	0	54	46
	0	59	41

* Values corrected for the presence of infranatant in supernatant, i.e. amount found in supernatant–volume of supernatant × concn in infranatant.

same behaviour as LPS with respect to the dissolution of testosterone, testosterone undecanoate, nandrolone phenylpropionate, lynestrenol acetate and dexamethasone-21-palmitate.

Since the LPS is used for the solubilization of steroid esters it was necessary to know whether LPS had hydrolytic activity for these esters. The esterase activity of LPS was measured by dissolving various nandrolone esters in LPS (concn: 100 μg/ml) and, after incubation of this solution at 37° for 16 or 21 hr, determination of the free nandrolone concentration in the solutions. No hydrolysis occurred in the case of the decanoate and palmitate esters while the acetate and propionate esters hydrolyzed to a small extent (1·5 and 5·7 per cent, respectively). The esterase activity is sufficiently low to permit use of freshly prepared steroid ester solutions in LPS.

Binding of steroids to lipoproteins

In order to demonstrate the binding of steroids to lipoproteins several experiments were performed. If there is a difference between the specific density of crystalline steroids and lipoproteins ultracentrifugation of steroid solutions in LPS at a carefully chosen density will separate undissolved steroid and steroid bound to lipoproteins. After the separation the quantities of bound and undissolved steroid can be determined.

Another method used to demonstrate the binding of drugs to proteins was equilibrium dialysis. A separation between bound and unbound steroid in LPS could also be achieved by electrophoresis of steroid solutions in LPS.

Steroid molecules have no charge and therefore they do not move during electrophoresis. Finally the solubilization of a steroid in the solid phase into lipoprotein solutions of various concentrations was investigated.

Ultracentrifuge experiments. Nandrolone, testosterone and testosterone undecanoate were dissolved in LPS in a concentration of 100 μg/ml. These solutions as well as solutions or suspensions in buffer were subjected to ultracentrifugation. This technique allowed a separation between the VLDL (in the supernatant), LDL (in the infranatant) and undissolved material (in the residue). The experiments with LPS were performed in duplicate and those with steroid solutions or suspensions in buffer were performed singly. The results of the analyses of the fractions are given in Table 2. Nandrolone is dissolved in buffer as well as in LPS; this is in contrast with testosterone which is only slightly soluble in buffer but to a much greater extent in LPS. Testosterone undecanoate is insoluble in buffer but completely soluble in LPS. The affinity of testosterone undecanoate for VLDL (supernatant) is much greater than that of testosterone and nandrolone. This is due to the greater lipophilicity of testosterone undecanoate.

Table 3. Equilibrium dialysis: the steroids were added to LPS inside the bag (I) or buffer outside the bag (II)

	I	II
Testosterone	44	32
Nandrolone	27	19

Values (means of duplicates) are in percentage binding.

Equilibrium dialysis experiments. These experiments were also performed with nandrolone, testosterone and testosterone undecanoate. Testosterone undecanoate could not pass the dialysis membrane. Neither testosterone nor nandrolone were bound to the membrane. Results of the experiments with nandrolone and testosterone are given in Table 3. Apparently equilibrium was not reached after 18 hr of dialysis. Nevertheless, the results demonstrated the existence of a binding of the two steroids to the lipoproteins. The binding of testosterone to the lipoproteins appeared a little stronger than that of nandrolone, which is in agreement with its slightly higher lipophilic character (see Table 1).

Solubilization of solid testosterone undecanoate by lipoprotein solutions. Finally divided testosterone undecanoate on pieces of filter paper was shaken either with lipoprotein solutions of different concentrations or with buffer. In Fig. 1 the dissolved quantity of testosterone undecanoate in lipoprotein solutions or in buffer is plotted against the time. In all cases the molarity of the buffer was the same (0·002 M Tris). Figure 1 shows clearly the much greater solubility of testosterone undecanoate in lipoprotein solutions when compared with its solubility in the buffer. It also shows that the maximum dissolution rate of solid testosterone undecanoate under these conditions (approx. 6 μg/ml per hr) is rather low.

Electrophoresis experiments. Solutions of [^{3}H]testosterone undecanoate in LPS and in pig serum were subjected to electrophoresis. The electropherograms were scanned and subjected to lipid staining. In the experiment with pig serum as solvent, an autoradiogram

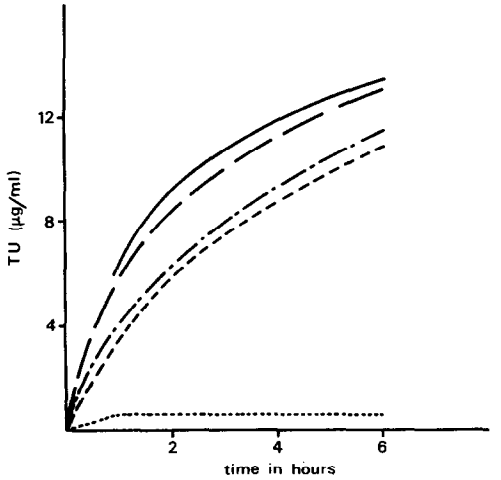

Fig. 1. Solubilization of solid testosterone undecanoate (TU) by lipoprotein solutions and the buffer. The abscissa shows the time in hours and the ordinate shows the dissolved amount of testosterone undecanoate in μg/ml. The molarity of the buffer was the same in all solutions. —— lipoprotein solution diluted 1:10; — — lipoprotein solution diluted 1:20; —·— lipoprotein solution diluted 1:40; - - - - lipoprotein solution diluted 1:60; ····· buffer (0·002 M Tris–HCl + 0·1% NaCl).

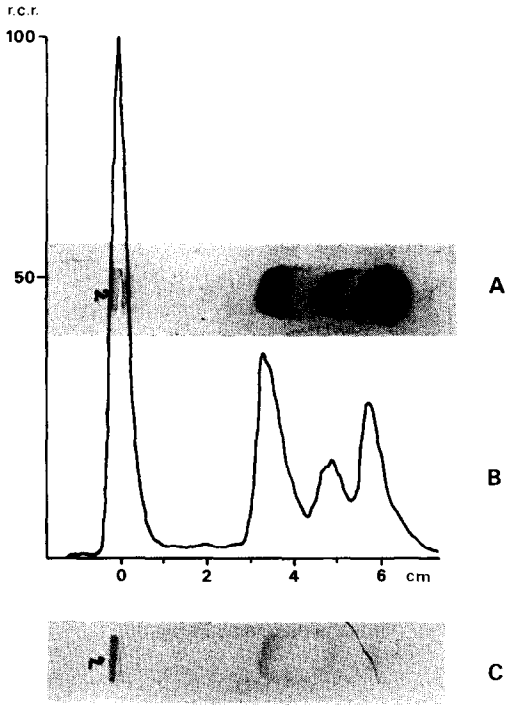

Fig. 2. Electrophoresis of pig serum, in which [^{3}H]testosterone undecanoate was dissolved (concentration 5 μg/ml). (A) Lipoprotein pattern of pig serum. (B) Distribution of radioactivity over the electropherogram (obtained by scanning). The abscissa shows the distance of the run in centimeters and the ordinate the relative counting rate (r.c.r.). (C) Autoradiogram of the electropherogram.

of the electropherogram was made before staining. Since the majority of the steroid dissolves in the usual fixing solution (5% acetic acid and 71% ethanol in water) the fixing solution used in these experiments was 5% acetic acid in water. A disadvantage of this mixture was that the gel easily detached from the glass plate, so careful handling was necessary. The steroid solution in pig serum (5 μg/ml) was stored at 20° for 1 hr before it was applied to the plate.

From the distribution of the radioactivity on the electropherogram and from the specific crescent shapes of the spots on the autoradiogram and the electropherogram after lipid staining as shown in Fig. 2, it is clear that a considerable fraction of the steroid dissolved in pig serum is bound to the lipoproteins. In another experiment the steroid was dissolved in LPS in a concentration of 20 μg/ml and immediately after preparation the solution was applied to the plate. From Fig. 3 it is clear that almost all of the steroid is bound to the lipoprotein. In a third experiment the effect of incubation on the binding of testosterone undecanoate to lipoproteins was studied. Aliquots of steroid solutions in LPS were applied immediately after the preparation, as well as after storing at 37° for one hour. The concentration of the steroid in LPS was 250, 500 and 1000 μg/ml. Figure 4 shows that when the steroid solutions in LPS were stored for one hour at 37°, the fraction of the steroid not firmly bound to the lipoproteins was greatly decreased. A possible explanation for this phenomenon is given in the discussion.

In vivo *experiments with testosterone undecanoate in rats*

During the foregoing experiments we had prepared solutions of lipophilic substances in an aqueous environment. In order to find out whether these solutions

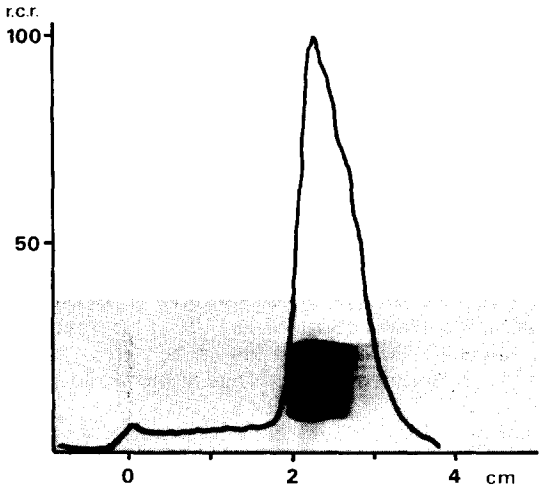

Fig. 3. Electrophoresis of LPS, in which [^{3}H]testosterone undecanoate was dissolved (concentration 20 μg/ml). Distribution of radioactivity over the electropherogram, together with the lipoprotein pattern after lipid staining. The abscissa shows the distance of the run in cm (0 = origin) and the ordinate the relative counting rate (r.c.r.).

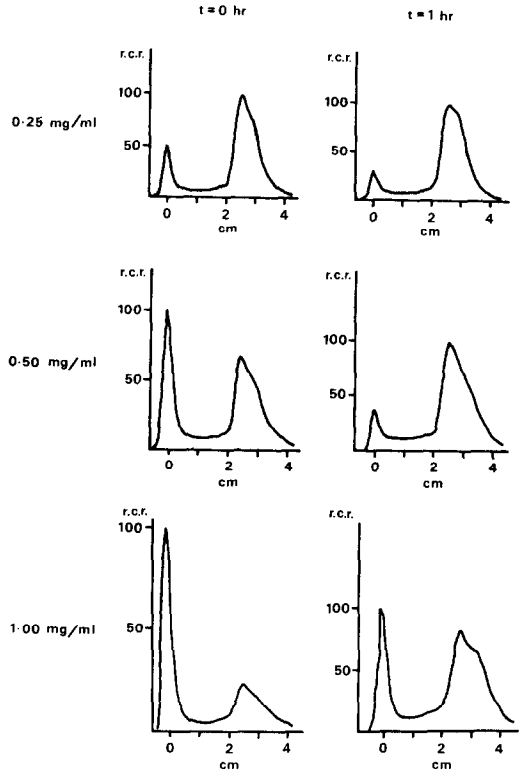

Fig. 4. The effect of incubation on the binding of testosterone undecanoate (TU) to lipoproteins. Testosterone undecanoate was dissolved in LPS in various concentrations and immediately or after incubation at 37° for 1 hr, subjected to electrophoresis. The abscissa shows the distance of the run (0 = origin) and the ordinate represents the relative counting rate (r.c.r.).

could be used for intravenous administration, two experiments were performed. In the first experiment the variation of the testosterone undecanoate concentration in blood with the time was determined after intravenous injection of this steroid in various solvents. In the second experiment the biological activity of testosterone undecanoate after intravenous administration in LPS was compared with its activity after subcutaneous administration in arachis oil.

Blood levels of testosterone undecanoate after intravenous administration in various solvents. Testosterone undecanoate was dissolved in LPS, in rat serum and in ethanol. The concentration of the steroid in LPS and in rat serum was 1 mg/ml and in ethanol 20 mg/ml. The solutions were injected intravenously in rats and the blood level of testosterone undecanoate was determined after various times. In all cases the injected amount of testosterone undecanoate was 1 mg. In Fig. 5 the steroid concentration in blood is plotted against the time. From Fig. 5 it is clear that the extrapolated concentration at zero time for the ethanol solution was approximately five times lower than that for the other two solvents. This is probably due to the rapid crystallization of testosterone undecanoate, presumably followed by sequestration of these micro-crystals in the capillaries when administered in ethanol.

Biological activity of testosterone undecanoate in the rat. The androgenic-anabolic activity of testosterone undecanoate, dissolved in LPS, was measured using the Hershberger test. One ml of the solution in LPS (concentration 0·5 mg/ml) was administered intravenously, daily for 7 days, and compared with a single injection of 1 ml of a testosterone undecanoate solution in arachis oil (concentration 1 mg/ml) on the first day. The results are given in Table 4. In both forms of administration testosterone undecanoate gave a good biological response.

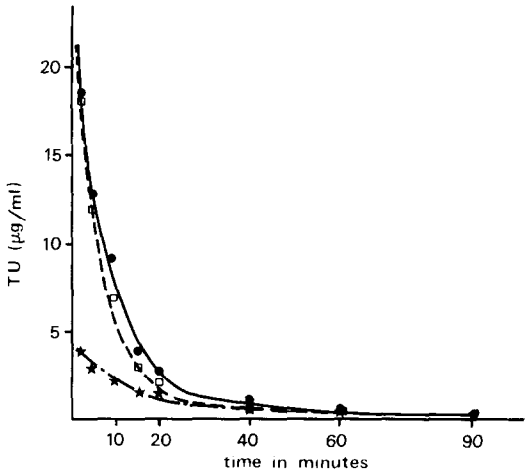

Fig. 5. Blood levels of testosterone undecanoate (TU) after i.v. administration of testosterone undecanoate in various solvents to the rat. The abscissa shows the time after injection in minutes and the ordinate shows the concentration of testosterone undecanoate in the blood in μg/ml. □——□ in LPS (concentration testosterone undecanoate 1 mg/ml); ●——● in rat serum (concentration testosterone undecanoate 1 mg/ml); *·–·–·* in ethanol (concentration testosterone undecanoate 20 mg/ml).

Table 4. Increase in weight of the seminal vesicles, ventral prostate and levator ani muscle (MLA) in percentage of control with the 90% confidence limits between brackets

Form of administration	No. of rats	Seminal vesicle	Ventral prostate	MLA
In LPS (i.v.)	6	255 (214–303)	390 (320–480)	145 (126–167)
In arachis oil (s.c.)	6	233 (196–287)	370 (300–440)	128 (111–147)

DISCUSSION

The existence of binding of steroids to lipoproteins has been shown by several experiments (ultracentrifugation, equilibrium dialysis, electrophoresis). The degree of binding of a substance to lipoproteins correlates well with the lipophilic character of that substance as shown by the solubilities of substances in LPS (Table 1) and, for some steroids, by ultracentrifugation and equilibrium dialysis experiments. This is also in agreement with the study of Kilian [18] who found a correlation between the absorption after oral administration of a substance via the lymphatic system and its lipophilic character, but it is not in agreement with the experiments of Avigan [1] who found a greater solubility of the more polar compounds in lipoprotein solutions. The relatively high solubility of nandrolone in LPS could be explained by its solubility in the buffer. The high solubility of nandrolone in the buffer in comparison with that of testosterone is surprising since there is only a small difference in polarity. However, the absence of one methyl group in nandrolone caused a gain of about 2 kcal/mole in the hydratation energy and this effect is probably responsible for the high solubility of nandrolone in buffer. Other serum proteins could also bind steroids and it is for this reason that the more polar steroids such as testosterone and nandrolone are also, or even preferably, bound to the other serum proteins. This is in agreement with the results of Rudman *et al.* [2], who found that several compounds such as testosterone, oestradiol and palmitic acid are partly bound to the lipoproteins in lipaemic sera. In our view the binding of lipophilic substances to lipoproteins proceeds in two steps. The first is the binding of a substance to the surface of the lipoproteins and in the second step the substance migrates to the very lipophilic environment of the innerside of the lipoproteins. The nature of the binding at the surface is most probably electrostatic because the surface consists of proteins and phospholipids, whilst the binding "inside" is hydrophobic in nature. In the latter condition the system has the lowest energy. The decreasing solubility of a substance in LPS when the ionic strength of the solution is increased supports this mechanism as do the results of the electrophoresis of steroid solutions which are shown in Fig. 4. The quantity of labelled material that remained at the origin might have been totally unbound or might have been part of the fraction that was bound at the surface of the lipoproteins, but dissociated from it by the interaction of the ions of the buffer employed in the electrophoresis. The former possibility is ruled out by the results of the experiment in which it was shown that the solubility of the steroid in buffer is approximately 0·6 μg/ml and the maximum dissolution rate of solid testosterone undecanoate is only 6 μg/ml per hr (see Fig. 1). In the experiment with a testosterone undecanoate concentration of 1000 μg/ml (Fig. 4) approximately 300 μg steroid per ml LPS was bound in 1 hr, and hence cannot be due to simple dissolution of the steroid. Because of these binding properties of lipoproteins LPS has proved to be an excellent solvent for lipophilic substances. Testosterone undecanoate, dissolved in LPS, retains its physicochemical properties and its biological activity after intravenous administration. Several investigators have proposed other solvents for steroids, such as albumin solutions [4, 19, 20], solutions of "association colloids" [21] and propylene glycol [22, 23].

Lipophilic substances, however, do not dissolve adequately in albumin solutions, "association colloids" (which consist of detergents) have toxic properties and propylene glycol causes haemolysis, thus making these solvents unsuitable for intravenous administration. However, a lipoprotein solution isolated from the same species to which it later has to be administered has no toxic properties and could dissolve appreciable amounts of steroids especially the very lipophilic ones. Moreover, solutions of lipophilic substances in lipoprotein solutions could be diluted many times without precipitation of the substance. For research purposes, such solutions would be suitable for intravenous administration.

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