DCA resulted in maximum serum progestin levels of 2 to 3 μg/ml. These concentrations are not significantly different from the values seen in the castrated control rats and definitely indicate that conversion of DCA to progestin occurred in adrenalectomized or nephrectomized rats. In animals deprived of both the adrenals and the kidneys, however, no detectable circulating progestin was found.

The experiments of Engelhart (*8*), who found that progestational changes could be induced in the uteri of young, unmated rabbits by subcutaneous injections of adrenocortical extracts, were probably the first suggestion concerning the possible elaboration of progestins by the adrenal gland of the mammal. Callow and Parkes (*9*) prepared extracts from the adrenals that caused full progestational changes in the rabbit's uterus, while Beall and Reichstein (*10*) were the first actually to isolate progesterone from the adrenal gland. The detection of circulating, progesterone-like activity in the male has been shown in the intact bird (*11*) and the intact or castrated mammal (*7*). In the case of the rat, increases in serum progestin have been reported within 6 hr following adrenal stimulation (*12*), and the titers of serum progestin present in the intact animal have been shown to disappear following adrenalectomy (*7*). Further evidence for the elaboration of progestin by the adrenal gland may be seen in the results of Lyons *et al.* (*13*) who produced deciduomata in the hypophysectomized, oophorectomized rat treated with ACTH. These facts purport an adrenal source of progestins in the male, but it is not known whether the hormone is secreted specifically by the adrenal gland or whether it results through conversion from other adrenal steroids, such as desoxycorticosterone.

Summary. The conversion of DCA to progestin *in vivo,* which has been shown in the case of the monkey (*4*) and now the rat, indicates one possible mechanism by which progestin may occur in the male. In addition, these studies also show that, at least in the rodent, the adrenals and the kidneys are the sites of this conversion.

Investigations on the conversion of DCA to progestin in other species, as well as studies on the release of progestin by tissue slices incubated with or without DCA, are currently in progress.

Table 1. Mean progestin levels in the serum of the rat following administration of 5 mg desoxycorticosterone acetate (DCA).

Hours after treatment with DCA	Serum progestin (μg/ml) in the rat			
	Castrated	Castrated and adrenalectomized	Castrated and nephrectomized	Castrated, adrenalectomized, and nephrectomized
2	2.00	1.00	1.50	0.00
4	3.33	3.00	2.00	.00
6	2.00	2.00	2.00	.00
12	1.00	1.00	1.00	.00
24	0.33	0.67	0.33	.00

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Light-Scattering Studies on Hyaluronic Acid

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Hyaluronic acid is an important constituent of many body tissues and occurs in synovial fluid, vitreous humor, and umbilical cord. This acidic polysaccharide is also present in some connective tissue and, as such, may take part in the physiological functions of connective tissue—that is, transport, storage, repair, and resistance to infection (*1*). In addition, it appears that there are physicochemical changes of the hyaluronic acid of joint fluids in rheumatoid arthritis (*2*). It is felt that a study of the size and shape of the molecule may contribute to an understanding of the role of this substance in its various physical functions. In the present work we are concerned with the results of light-scattering determinations. Other physicochemical studies are in progress.

Ogston and Stanier (*3*) have studied raw ox-synovial fluid and its ultrafiltrate containing hyaluronic acid. The material in the ultrafiltrates contains approximately 30 percent protein with the hyaluronic acid. By means of flow birefringence and viscosity studies, Ogston and Stainer concluded that the particles are highly hydrated spheres and that under the influence of a shear gradient the particles are deformed.

Light scattering affords a means whereby the size and shape of the particles in solution are determined without subjecting the particles to any external stress and where the theoretical interpretation of the experimental data is explicit.

Hyaluronic acid was prepared in the laboratory of Karl Meyer from umbilical cord by alcohol fractionation after peptic and tryptic digestion (*4*). Chemical analysis of the material gave the following results in percentages: nitrogen, 3.03; hexosamine, 38.0; sulfate, less than 0.4; hexosamine/nitrogen, 0.98; uronic acid (Dische method), 45.5. Our starting solutions were 0.5 percent by weight of hyaluronic acid in a 0.10*M* acetate buffer medium at *p*H 5.0 containing 0.15 molar sodium chloride. The solutions were clarified by centrifugation in a clinical centrifuge at 5000 rev/min for 20 min. The buffer solution used for dilution was clarified by filtering through a fine-grade sintered glass filter and by centrifugation.

Measurements were made in the Aminco Light Scattering apparatus (*5*) using the green line of mercury (λ, 546 mμ) throughout. A micro light-scattering cell was used that required approximately 3 ml of solution (*5*). This cell was surrounded by a larger cylindrical cell containing the solvent to decrease the reflectivity.

The refractive index increment of the solutions $(n-n_0)/c$, where n_0 is the refractive index of the solvent and n is the refractive index of the solution at concentration c (g/ml), needed in the molecular-weight determinations was measured in a Rayleigh interferometer. It was found to equal 0.180 independent of the concentration.

Information from light scattering concerning the size, shape, and molecular weight is gained from two sources, the dissymmetry and the turbidity (*6*). The observed values of the angular scattering given in terms of the dissymmetries (intensity at angle θ divided by the intensity at angle $180°-\theta$, where θ is measured from the transmitted beam) were 5.65, 5.32, 4.77, 3.40, 2.39, and 1.53 for θ equal to 40°, 45°, 50°, 60°, 70°, and 80°, respectively. The dissymmetries were found to be practically independent of concentration from 0.125 to 0.5 percent. The scattering for the solution is greater in the forward directions ($\theta < 90°$) than in the backward directions ($\theta > 90°$). Theory indicates (*6*) that the particles are not rigid rods, since $I_{45°}/I_{135°}$ cannot exceed 2.4, no matter what the length of the rod may be. From theoretical considerations for a random coil (in the high-polymer sense) and a sphere, graphs have been constructed of the dissymmetry for various pairs of supplementary angles, $I_\theta/I_{180°-\theta}$, versus L/λ', where L is the largest dimension of the particle and λ' is the wavelength of the light in the medium; $\lambda'=\lambda/n_0$, where n_0 is the refractive index of the solvent and λ the wavelength of the incident light. The observed values in the present study are incompatible with the theoretical values calculated for a random coil but are all in close agreement with those expected for a sphere where $L/\lambda'=0.51$. Hence we conclude from the dissymmetry measurements that the particles are spheres of diameter $L=546\times0.51/1.33=210$ mμ.

From turbidity measurements, we obtain the molecular weight of the material having a different refractive index from that of the solvent—that is, the "dry" hyaluronic acid. To determine the turbidity, the observed intensity of scattering at 90° was related to the absolute turbidity by the use of Ludox whose absolute turbidity is measured by transmission measurements (*5*). The turbidity at 90° must be multiplied by a factor to account for the lack of symmetry of the scattering envelope (*6, 7*), and this factor is calculated from our observed dissymmetry values to be 2.90. The turbidity divided by the concentration was found to be independent of concentration over the range studied (0.125 to 0.500 percent), although there is evidence for interaction at concentrations higher than these. The solutions that we studied may therefore be regarded as thermodynamically ideal.

For ideal solutions the molecular weight M is given (*6*) by

$$M=\frac{\tau}{Hc}, \text{ where } H=\frac{32\pi^3 n_0^2}{3N\lambda^4}\left(\frac{n-n_0}{c}\right)^2;$$

N is the Avogadro number and τ is the turbidity corrected for the dissymmetry. Since $\lambda=546$ mμ and for hyaluronic acid $(n-n_0)/c$ is 0.180, then $H=3.54\times10^{-6}$. In our determinations the average value of Hc/τ over the concentration range studied was 1.26×10^{-7} and therefore $M=8\times10^6$.

The dissymmetry measures the outline of a molecule and gives a length corresponding to its greatest dimension. The turbidity gives a value for the molecular weight of the "dry" material present. The molecular weight that we obtained from the corrected turbidity is considerably less than that expected for a sphere of diameter 210 mμ determined from the dissymmetry. It appears, therefore, that the particles are highly swollen spheres of diameter 210 mμ and that the hyaluronic acid contained within the sphere has a molecular weight of 8.0×10^6. Simple calculations show that such spheres contain 0.27 percent by weight of anhydrous hyaluronic acid, and the remainder is presumably water or solvent. Hence the "swelling" is considerable.

It is interesting that the molecular model arrived at by our methods for an essentially protein-free hyalutonate resembles to a great extent the model of Ogston and Stanier (*3*) who used a hyaluronic-protein complex. This finding might indicate that the protein does not contribute to the general shape and physical properties of the molecule.

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References and Notes

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