tion and cytopathogenic changes. Sensitivity equaled that of monkey kidney, both with stock cultures of virus and stool specimens from patients. Stool "toxicity" was no more troublesome than in monkey kidney. A marked difference between monkey kidney and amnion occurred in the Coxsackie B series. the susceptibility of amnion resembling that seen with HeLa cell cultures(5,6). Only type B-3 produced cytopathogenic effects in amnion, while 2, 3, and 4 did so in monkey kidney. Types B-1 and B-5, which are known to cause degeneration in monkey kidney(7) were not tested. Of the Coxsackie A group, only type A-9 has been studied, and this produced cytopathogenic changes both in monkey kidney and in human amnion. Strains of Influenza A and B, which caused cellular changes in monkey kidney, failed to produce similar changes in amnion. Hemagglutination studies of the supernatant were not performed. Herpes simplex and Western Equine Encephalitis viruses also produced cellular degeneration in both types of tissue. No "wild" viruses have appeared in amnion cultures.

Summary. 1) A simple, reliable, and reproducible method has been developed to grow monolayer cultures of human amnion. 2) Placentas from routine vaginal deliveries are used without modifying patient preparation or care. 3) Unminced, washed amnion is trypsinized at room temperature, and the resulting cell suspension is inoculated into bottles or tubes. The best results are obtained by first growing the cells in large bottles and transferring them to tubes. 4) Susceptibility of human amnion tissue cultures to a variety of viruses has been studied, and the results are reported.

1. Zitcer, E. M., Fogh, J., and Dunnebacke, T. H., *Science,* 1955, v122, 30.

2. Fogh, J., and Lund, R. O., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 80.

3. Hok, K. A., 1955, personal communication.

4. Dulbecco, R., and Vogt, M., *J. Exp. Med.,* 1954, v99, 183.

5. Crowell, R. L., and Syverton, J. T., *J. Immun.,* 1955, v74, 169.

6. Sickles, G. M., Mutterer, M., Feorino, P., and Plager, H., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 529.

7. Sickles, G. M., Feorino, P., and Plager, H., *ibid.,* 1955, v88, 22.

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Plasma Amino Nitrogen and Creatine Values of Growing Chick and Laying Hen.* (22536)

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A search of the literature reveals few reliable standard values for amino nitrogen and creatine in chicken blood. Sturkie(1) does not present any amino nitrogen values in his summary of the chemical constituents of chicken blood, nor does Albritton(2) in his monumental reference on standard blood values. Edwards and Wilson(3) reported a higher blood amino nitrogen level in the non-fasted laying hen under conditions of hyperthermy. Hsu and Combs(4) noted that a vit. B_{12} deficiency of 4-week-old chicks resulted in significantly higher blood nitrogen compounds, including amino nitrogen. There is considerably more information in the literature on creatine(1,2) than on amino nitrogen values for chicken blood, but these determinations were made with alkaline picrate and without due correction for non-creatinine compounds that give a positive Jaffe reaction.

Methods. In this laboratory plasma cre-

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TABLE I. Representative Values for Creatine and Amino Nitrogen in Chicken Blood.

	Creatine,* mg/100 ml plasma†	Amino nitrogen, mg/100 ml plasma
	5-wk-old cockerels‡	
	.83	8.74
	.92	8.52
	1.08	8.30
	.50	8.16
	.83	7.96
	.67	8.30
	1.17	7.96
	1.58	9.28
	1.08	7.20
	1.08	9.28
Avg ± S.D.	.97 ± .09	8.37 ± .40
	Laying hens	
	1.5	8.10
	1.0	7.42
	.92	8.10
	.75	8.10
	1.25	7.76
	1.83	8.40
	1.33	7.64
	.92	7.96
	1.42	8.30
	.74	7.96
Avg ± S.D.	1.17 ± .13	7.97 ± .09

* Measured as total creatinine; preformed creatinine was not measurable.

† Assuming a hematocrit of 30%, plasma values can be converted to whole blood values by multiplying by 0.7; avg values are .69 ± .05 and .82 ± .06 for cockerels and hens respectively.

‡ Differences between chicks and hens are not significant.

atine and amino nitrogen are now being determined as part of a routine clinical procedure. Plasma creatine is determined by the method of Owen, *et al.*(5). After conversion of creatine to creatinine, Lloyd's reagent in acid solution is used to absorb selectively the plasma creatinine; subsequently, it is eluted from the silicate by alkaline picrate, thus eliminating the presence of materials that would give a positive Jaffe but false creatinine reaction. Amino nitrogen is determined by the naphthoquinone method of Danielson as presented by Hawk, *et al.*(6). Representative values from the plasma of growing chicks and laying hens are shown in Table I.

Results. Edwards and Wilson(3) using the copper method of Albanese and Irby(7) reported values of 7-12 mg of amino nitrogen per 100 ml blood. Hsu and Combs(4) employing the naphthoquinone method reported amino nitrogen values of 20-25 mg/100 ml blood. Both investigators used a Folin-Wu tungstic acid protein-free plasma filtrate for their determinations yet reported their values in terms of whole blood. The report of Hawk, *et al.*(6) that red blood cells have a significantly higher content of amino nitrogen than the plasma, emphasizes the importance of properly presenting amino nitrogen contents in terms of the specific blood fraction analyzed. Another source of error often encountered if values are presented in terms of whole blood is the hematocrit. The hematocrit for chick and hen blood (but not for adult roosters) is 28-30(8) in contrast to the value of 47-50 of man and most mammals(2). Thus, if not taken into account, the hematocrit conversion factor can involve an appreciable error. The representative values for amino nitrogen in chicken plasma presented in Table I fall within the range reported for human plasma(6). The high values reported by Hsu and Combs have not been confirmed in this laboratory. The blood creatine values reported by Sturkie(1) range from 0.8-1.8 mg/100 ml blood with only 2 of 10 values falling below 1 mg/100 ml. These values are generally higher than those found in this study in which Lloyd's reagent was employed to selectively absorb creatinine. Comparisons obtained with and without Lloyd's reagent on the same sample of plasma invariably gave significantly higher readings in the absence of Lloyd's reagent. This corroborates the findings of Owen, *et al.*(5) of the presence of non-creatinine materials in chicken and human blood that give a positive Jaffe reaction.

Summary. Representative values for amino nitrogen and creatine are given for chicken blood. Creatine values previously reported in the literature appear to be too high because of non-creatine chromogens.

1. Sturkie, P. D., *Avian Physiology,* Comstock Publishing Associates, Ithaca, N. Y., 1954.

2. Albritton, E. C., *Standard Values in Blood,* W. B. Saunders Co., Philadelphia, 1952.

3. Edwards, W. H., and Wilson, W. O., *Am. J. Physiol.,* 1954, v179, 76.

4. Hsu, Peng-tung, and Combs, G. F., *Arch. Biochem. Biophys.,* 1952, v38, 29.

5. Owen, J. A., Iggo, B., Scandrett, F. J., and Stewart, C. P., *Biochem. J.*, 1954, v58, 426.

6. Hawk, P. B., Oser, B. L., and Summerson, W. H., *Practical Physiological Chemistry*, 13th edition, the Blakiston Co. Inc., New York, 1954.

7. Albanese, A., and Irby, V., *J. Biol. Chem.*, 1944, v153, 583.

8. Sturkie, P. D., and Newman, H., *Poultry Sci.*, 1951, v30, 240.

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Fractions of Commercial Pitressin which Release ACTH.*‡ (22537)

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Commercial Pitressin and certain fractions derived from it by chromatography, acid hydrolysis, Craig counter current distribution and other methods, are active ACTH releasing agents(1-9). In a footnote to an earlier communication(7), the writers called attention to the marked ACTH-releasing activity of hydrolysates of pitressin (Vasopressin Injection, Parke, Davis and Co.), in which the pressor activity had been destroyed by boiling for 1.5 hours in 2.2 N HCl. In addition to the releasing factor the acid hydrolysates contain a component indistinguishable in its activity from histamine when tested on the isolated ileum of the guinea pig and blood pressure of the etherized-atropinized cat.

Methods. A simple modification of the method of Saffran, *et al.*(5,6), was employed for determining the ACTH-releasing potency of pitressin† and its various fractions obtained by acid hydrolysis and Craig counter current distribution. Halved anterior pituitaries of adult rats were incubated in the Warburg apparatus for one hour with 0.67 mg of the test material. Small amounts of the incubation fluid (0.20 cc diluted to 0.5 cc), were then infused i.v. into 120-140 g male rats hypophysectomized 18-24 hours previously for adrenal ascorbic acid assay, as a measure of the efficacy of the test material for releasing ACTH.

Results. Table I-A gives the pertinent data obtained from experiments on the ACTH-releasing substance remaining in the pitressin hydrolysates after all pressor activity had been destroyed by boiling in strong acid(7).

Hydrolyzed pitressin exhibits potent releasing activity when incubated with halved pituitary glands *in vitro*, since the fall in adrenal ascorbic acid of the hypophysectomized rats averaged well over 100 mg %. The control halves of the same pituitary glands generally released small amounts of ACTH when compared with the quantities released by the experimental series. Arterenol added to the incubation fluid as originally recommended by Saffran, *et al.*, does not enhance releasing activity (Table I,A); therefore, it was not used in subsequent experiments. The hypophysectomized rat shows no depletion of adrenal ascorbic acid when infused i.v. with 300 units of pitressin boiled in 2.2 N HCl for 1.5 hours, despite the fact such material is highly active in releasing ACTH from pituitaries incubated in the Warburg.

Histamine, equivalent to 0.22 μg of the base, when added to incubation fluid containing halved pituitary glands did not induce release of greater amounts of ACTH than the corresponding control glands. These data agree with other reports of the ineffectiveness of this amine as an ACTH releaser(3,4) and make it unlikely that the histamine-like sub-

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