Summary. Seven alkyl acid phosphates have been found able to solvate amino acids in dry acetone. Marked differences in solvating power are shown by these reagents for individual amino acids, and for selective solvation of amino acids from complex mixtures such as a protein hydrolysate. Mixtures of amino acids greatly influence each others' solubility when fractionation is effected by the method described.

When systems consisting of acetone-amino acid-solvating reagent, are neutralized with triethylamine, amino acids are precipitated, but never quantitatively. Marked differences occur in the extent to which the dissolved amino acids precipitate with this treatment; *e.g.* 90% were precipitated from the n-propyl, as against but 45% from the isopropyl system, when the amino acids were solvated from a dry casein hydrolysate.

These reagents offer prospects for usefulness in the fractionation of amino acid mixtures.

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The Cardiac Output and Circulation Time of Ferrets.* (17552)

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The ferret was first used as a laboratory animal by Dunkin and Laidlaw for the study of canine distemper.(1) Subsequently it was also found to be susceptible, under experimental conditions, to other viral infections such as influenza,(2) fowlpox,(3) swineherd's disease,(4) Rift Valley fever,(5) a strain of poliomyelitis virus,(6) and lymphocytic chorio-meningitis.(7) In influenzal infection, experimental investigations employing ferrets have contributed a great deal to our knowledge of the pathology(8) and epidemiology(9) of this important disease. Other aspects of the infection, such as the problem of the toxicity of the virus and the alterations in the infected host's physiological functions are fields relatively unexplored. It seems that the ferret, because of its larger size, might serve better than the mouse or other rodents for investigations of these problems. Except for a general description by Pyle,(10) we are not aware of any detailed report dealing with the normal physiology of this animal. The present study dealing with the cardio-vascular function of normal ferrets was initiated in order to obtain such data before investigations of pathological physiology were undertaken. Cardiac output determined by the direct Fick principle, arterial blood pressure, and circulation time measurements were selected to depict the status of the animal's cardio-vascular functions.

Materials and methods. 1. *Animals*: North American ferrets (*Putorius putorius*)‡ of both sexes with body weights varying from 700 to 1100 g were used. The body surface was calculated by the formula:(11) Surface area in square meters = (body weight in g)$^{2/3}$ ×

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1. Dunkin, G. M., and Laidlaw, P. P., *J. Comp. Path.*, 1926, v39, 201.

2. Smith, W., *et al.*, *Lancet*, 1933, v2, 66.

3. Findlay, G. M., and Mackenzie, R. D., *Brit. J. Exp. Path.*, 1937, v18, 146.

4. Durand, P., *C. R. Acad. Sc.*, 1936, v203, 957.

5. Francis, T., and Magill, T. P., *J. Exp. Med.*, 1935, v62, 433.

6. Patocka, F., *Zbl. Bact. Abt. I Orig.*, 1941, v148, 15.

7. Dalldorf, G., *J. Exp. Med.*, 1939, v70, 19.

8. Perrin, T. L., and Oliphant, J. W., *Public Health Rep., Wash.*, 1940, v55, 1077.

9. Commission on Acute Respiratory Diseases, *Am. J. Hygiene*, 1948, v48, 292.

10. Pyle, N. J., *Am. J. Public Health*, 1940, v30, 787.

‡ Identified by Mr. K. P. Schmidt, curator of zoology, Natural History Museum, Chicago.

1/1000. The validity of the calculated value was carefully checked by actual measurements on the animals.

2. *Circulation time.* The experiments were performed in the morning on fasting animals. Anesthesia was induced by intraperitoneal injection of a solution of sodium nembutal, 30 mg per kg of body weight. Ten minutes later, the animal was placed on a board in supine position. Circulation time was then determined by the use of two technics: (a) A short incision was made in the left inguinal region and the femoral vein isolated. Five-tenths ml of a 10% sodium fluorescein solution was quickly injected into the vein. The interval between the beginning of the injection and the appearance of a green fluorescence in the oral mucous membrane, observed under ultra-violet light, was accurately timed; (b) A superficial vein in the neck was isolated and two-tenths ml of a 1% sodium cyanide solution was injected. The time from the beginning of the injection and the appearance of a deep inspiratory movement was noted.

3. *Cardiac output.* An incision was next made in the midline of the neck exposing the trachea for cannulation. The left common carotid artery was freed and cannulated with a No. 20 needle with a blunt bevel, connected to a mercury manometer which registered on a kymographic drum. The external jugular vein on the opposite side was dissected free and its cephalid end was tied. With the use of a specially constructed forceps(12) having sharp thin tips, an opening was made and held open in the wall of the vein. A No. 025 polythene catheter with metal stylet was introduced into the vein between the opened forceps. The catheter was advanced carefully toward the heart. The final position of the tip of the catheter was ascertained by placing the animal under a fluoroscope and with further manipulations the tip was made to lie in the right ventricle. The stylet was then removed and 0.2 ml of a 10% heparin solution in saline was introduced into the catheter to prevent clotting. The position of the catheter was fixed by tying a ligature around the vein.

The cardiac output was determined according to the direct Fick principle.§ The oxygen consumption was measured by connecting the tracheal cannula to a spirometer containing 100% oxygen. The volume of oxygen consumed was readily calculated from the kymographic tracing. As soon as the tracheal cannula was linked to the spirometer, a 1.5 ml sample of arterial blood was withdrawn from a three-way stopcock which was connected to the common carotid artery for the blood pressure determinations. A sample of venous blood (1.5 ml) was then quickly drawn from the polythene catheter. Both samples of blood were drawn into paraffined syringes and delivered immediately under a layer of paraffin oil in tubes. Determinations of the oxygen contents of these samples were made without delay according to the method of Van Slyke and Neil.(13) Five-tenths ml samples of blood were used for each determination and duplicates checked within 0.1 vol. %. Before the animal was killed, 1 ml of venous blood was taken from the polythene catheter, heparinized, and hematocrit readings were made by Wintrobe's method.

Results. Our findings (Table I) on the average rectal temperature and respiratory rate among the 10 normal animals were lower than those reported by Pyle(10) chiefly because our readings were made on anesthetized animals. Ferrets were easily excitable animals and such readings taken on unanesthetized animals were subject to wide fluctuations. The cardiac output, expressed in the form of cardiac index, was calculated in terms of liters per square meter of body surface per minute and was on the average 2.476 $\pm$ 0.75 liters. The average oxygen content of arterial blood was 15.7 vol. % and that of venous blood 10.2 vol. %. The average mean arterial blood pressure was 147

11. D'Amour, F. E., and Blood, F. R., Manual for Lab. Work in Mammalian Physiology, Exp. No. 26, Univ. Chicago Press, 1948.

12. Haas, E., and Goldblatt, H., *Science*, 1948, v108, 166.

§ Cardiac output =

$$\frac{\text{Oxygen consumption in ml per minute}}{\text{(Arterial O content) — (Venous O content)}}$$

13. Peters and Van Slyke, *Quantitative Clinical Chemistry*, Vol. 2, p. 321.

TABLE I. Measurements of Circulatory Function of Normal Ferrets.

No.	Sex	Body wt, g	Rectal temp., °F	Respiratory rate, per min.	Pulse rate, per min.	O consumption, cc/min.	O content arterial blood vol., %	O content venous blood vol., %	A-V O difference, vol., %	Cardiac output, cc per min.	Body surface (m^2)	Cardiac index (liter/min./m^2)	Blood pressure, mm Hg	Fluorescein circulation time, sec.	Cyanide-circulation, sec.	Hematocrit, %
1	M	730	100	27	246	7.2	16.3	8.1	8.2	87.8	.052	1.68	120			
2	F	800	101.4	13	248	6.0	15.8	10.6	5.2	115.4	.055	2.09	156			
3	F	850	100.2	14		7.5	20.4	12.8	7.6	98.7	.057	1.75	138			
4	M	780	100.8	28	168	5.5	17.9	11.2	6.7	82.0	.054	1.51	134	9.0	3.9	49
5	M	1120	99.8	26	290	10.4	15.4	9.5	5.9	176.2	.067	2.61	185	8.1	3.1	35
6	M	800	99.3	21	168	3.8	18.7	15.5	3.2	118.7	.055	2.15	175	6.5	5.5	46
7	M	800	100.8	20	278	10.8	13.5	8.1	5.4	200.0	.055	3.62	133	4.5	4.9	41
8	F	800	98.0	32	250	7.9	13.2	8.1	5.1	154.9	.055	2.81	150	5.8	5.2	32
9	F	700	97.3	38	172	3.9	12.4	9.9	2.5	156.0	.051	3.06	150			41
10	M	850	97.0	58		9.6	13.2	8.3	4.9	195.9	.057	3.42	136			33
Mean		823	99.4	28.6	227	7.2	15.7	10.2	5.5	138.5		2.467	147	6.8	4.5	40
Stand. dev.		114	1.5	13.1	50.5	2.5	2.6	1.8	**1.7**			0.753	20	1.2	0.7	4.7

mm of mercury.

Circulation time measured by the fluorescein technic (6.8 sec.) was longer than that determined by the cyanide method (4.5 sec.), because of the greater distance the dye had to traverse when it was injected through the femoral instead of the superficial neck vein. The cyanide technic gave a sharper endpoint, although it has the objection of including the reaction of the respiratory center in a function test primarily for the circulatory system. The short circulatory time of ferrets rendered it very likely that technical errors such as the rate of injection and promptness in perceiving the endpoint would jeopardize the reliability of the results.

Discussion. In this group of animals, all the common conditions known to alter cardiac output as ingestion of food, hemorrhage, exercise and oxygen content of the inspired air(14) had been eliminated or made as uniform as possible. The only uncontrollable factor was the individual variations in response to the effect of the anesthetic used. In dogs, Shore(15) had shown that the effect of barbituric anesthesia on cardiac output was quite variable during the course of their experiments. The differences between successive determinations on the same animals were as high as 35%. Furthermore, anesthesia might cause hypoventilation or atelectasis, both of which would interfere with normal cardiac function. The individual variations in the cardiac output of our normal animals were partly technical and partly real. Our results agreed with those of Warren *et al.* on human subjects(16) in that arterial blood oxygen showed less variable results, provided that hematocrit readings were fairly constant. Greater degrees of variations, however, were encountered in the measurements of oxygen consumption. Because of these variations, they concluded that, "results are valid only when groups rather than individuals are studied."

14. Whitehorn, W. V., *et al.*, *Am. J. Physiol.*, 1946, v146, 61.

15. Shore, R., and Knoefel, P. K., *Am. J. Physiol.*, 1945, v143, 709.

16. Warren, J. V., *et al.*, *Am. J. Physiol.*, 1945, v145, 458.

In a table compiled by Marshall(17) the cardiac indices for various animals were: Dog, 2.86; goat, 3.07; rabbit, 1.69; and horse, 5.84. The mean cardiac index of 2.476 for the present group of normal ferrets is one intermediate between those for dog and rabbit.

Summary. The mean cardiac output per minute per square meter of body surface for a group of 10 normal ferrets was found to be 2.476 liters. The average circulation time by the fluorescein technic was 6.8 seconds and 4.5 seconds as determined by the cyanide method. The average mean arterial blood pressure was 147 mm Hg. Various other related data were also tabulated.

17. Marshall, E. K., *Harvey Lectures*, 1929, v25, 64.

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Amount of Alkaline Phosphatase in the Oviduct of Folic Acid Deficient Chicks.* (17553)

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Increases in alkaline phosphatase in the reproductive tract following injection of estrogens have been described for many species. Hertz and Sebrell(1) first noted an impairment in the response to stilbestrol of the oviduct of chicks deficient in folic acid. This failure of estrogens to induce growth in the absence of folic acid has also been observed in the monkey,(2) frog,(3) and rat.(4) The following experiments were undertaken to determine whether the amount of alkaline phosphatase in the oviduct of the chick is affected by a folic acid deficiency.

Material and methods. Exp. 1. Eleven white leghorn chicks were obtained at hatching and placed in cages having elevated wire mesh floors. At 2 days of age 5 chicks were placed on a diet of chick mash, while the remainder were fed chick mash containing 2% folic acid antagonist.‡ Both groups received water *ad libitum*. Three birds in each group were kept as uninjected controls. The remaining chicks were injected subcutaneously with 50μg of estradiol§ in 0.05 ml of sesame oil daily for 5 days. The injections were started on the 12th day of feeding and the chicks were killed 24 hours after the last injection. At post mortem the oviducts were removed, weighed and placed in cold 80% ethyl alcohol. Histochemical studies for alkaline phosphatase were carried out by the method of Gomori,(5) in which the sections were incubated at 37°C for 2 hours with glycerophosphate and examined without the use of a counterstain. Body weights were recorded daily and blood samples obtained at autopsy for hemoglobin determinations.

Exp. 2. A total of 38 white leghorn chicks were obtained at time of hatching and treatment started at 2 days of age. Thirteen of the chicks received the normal diet, 13 received the normal diet containing 2% folic acid antagonist and 12 received a synthetic

* Aided in part by a grant from the American Cancer Society, Inc., to Harvard University.

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1. Hertz, R., and Sebrell, W. H., *Science*, 1944, v100, 293.

2. Hertz, R., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 113.

3. Goldsmith, E. D., Schreiber, S. S., and Nigrelli, R. F., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 299.

4. Hertz, R., and Tullner, Wm. W., *Endocrinol.*, 1949, v44, 278.

‡ We are indebted to Dr. E. L. R. Stokstad, Lederle Laboratories, New York, for the crude x-methyl folic acid used in these studies.

§ Estradiol was obtained through the courtesy of the Schering Corporation, Bloomfield, N.J.

5. Gomori, G., *J. Cell. and Comp. Physiol.*, 1941, v17, 71.