optima, one at acid and one at alkaline pH. This corresponds to what was found for liver pyrophosphatases by Bo NORBERG[3]. Both optima of articular cartilage and epiphyseal cartilage plate are slightly nearer to neutrality than those of bone and marrows. The absolute amounts of orthophosphate produced by the various bone constituents are quite different. The lowest activity is shown by the epiphyseal cartilage plate, although the presence of a pyrophosphatase is clear. This is in agreement with PERKIN's[4] observation that in rabbits the pyrophosphatase activity in the epiphyseal plate is lower than in the metaphysis. We therefore cannot confirm the data of CARTIER and PICARD[1] who found no pyrophosphatase in the epiphyseal cartilage plate. Moreover, since a considerable activity in the newly formed bone of metaphysis has also been found by these authors, their negative finding in cartilage, unless due to the different age of the animals used (sheep embrios in their experiments), is probably to be ascribed to a lower sensitivity of the methods they employed. A moderate increase of activity in compact bone and the two spongiosas was observed when $MgCl_2$ was omitted from the incubation medium. This result agrees with LIEBKNECHT's[5] data on bone phosphatase.

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Riassunto

Si è studiata l'attività pirofosfatasica, determinandone il pH ottimale, in 9 frazioni scheletriche istologicamente uniformi: cartilagine articolare e cartilagine di coniugazione, osso spongioso epi e metafisario, osso compatto, midollo diafisario, midollo dell'osso spongioso epifisario e dell'osso spongioso metafisario, periostio.

[3] Bo NORBERG, Acta chem. scand. *5*, 325 (1951).
[4] H. R. PERKINS, Biochem. J. *57*, XV P (1954).
[5] W. L. LIEBKNECHT, Biochem. Z. *303*, 96 (1939).

Serum Cholesterol Levels in Germ-Free Chickens

The major products of cholesterol catabolism are the bile acids[1]. The role of intestinal bacteria in the turnover of bile acids[2] plus the findings that, under certain conditions, sulfa drugs[3] or antibiotics[4] may alter serum cholesterol levels in cholesterol-fed animals has led us to investigate the serum cholesterol levels of germ-free chickens as a base line for subsequent work.

White Leghorn Chickens were used. They were reared by our own modification[6] of the method of REYNIERS *et al.*[6]. 18-day old embryonated eggs held in a nylon net were immersed for 2 min in a 0·15% (W/V) detergent solution[7] and then for 12 min in a 2% mercuric chloride solution at 37°C. From this bath the eggs were transferred through a tube without exposure to nonsterile air into a previously sterilized germ-free unit. Control eggs from the same hatch were treated in a similar fashion before being transferred to an incubator. Both groups of chicks, germ-free and conventional, were fed an autoclaved semisynthetic diet[8]. The germ-free chickens were given canned sterilized water[9,5] while the conventionally reared birds were given tap water.

The sterility of the chickens in the germ-free units was determined by taking frequent fecal samples and inoculating them into various bacteriological media according to a standard procedure which has been described previously[5].

At the termination of the experiment, the chickens were killed by exsanguination under ether anesthesia and blood samples taken by heart puncture. Sera were analyzed for cholesterol by the method of Trinder[10].

Two groups of birds aged 6 and 8 weeks respectively were studied and the average results (with standard deviations) are tabulated in the Table.

It is evident that while the germ-free chickens showed a better growth rate than the controls confirming earlier findings[5], there were no significant differences in the serum cholesterol levels of the two groups. These data

[1] M. D. SIPERSTEIN and A. W. MURRAY, J. clin. Invest. *34*, 1449 (1955).
[2] B. E. GUSTAFSSON, S. BERGSTROM, S. LINDSTEDT, and A. NORMAN, Proc. Soc. exp. Biol. Med. *94*, 467 (1957).
[3] O. W. PORTMAN, E. Y. LAWRY, and D. BRUNO, Proc. Soc. exp. Biol. Med. *91*, 321 (1956).
[4] D. KRITCHEVSKY, W. C. GRANT, M. J. FAHRENBACH, B. A. RICCARDI, and R. F. J. MCCANDLESS, Arch. Biochem. Biophys. *75*, 142 (1958).
[5] M. FORBES and J. T. PARK, J. Nutrition, in press.
[6] J. A. REYNIERS, P. C. TREXLER, R. F. ERVIN, M. WAGNER, T. D. LUCKEY, and H. A. GORDON, Lobund Reports *2*, 70 (1949).
[7] PN-700 Conditioner, Service Industries, Phila., Pa.
[8] Diet (per 100 g): Cornstarch, 57·0 g; Casein (purified), 25·0 g; Corn oil, 5·0 g; Alphacel, 3·0 g; Glycine, 1·5 g; L-Arginine-HCl, 1·0 g; DL-Methionine, 0·5 g; Choline-HCl, 0·27 g; Thiamine-HCl, 0·1 g; Ca Pantothenate, 10·0 mg; Nicotinic acid, 10·0 mg; Riboflavin, 4·0 mg; Pyridoxine HCl, 2·0 mg; Folic acid, 1·0 mg; Vitamin B_{12}, 0·005 mg; Biotin, 0·1 mg; Menadione, 0·8 mg; Vitamin A, 2,600 IU; Vitamin D_3, 100 ICU; a-Tocopherol, 5 mg; $CaCO_3$, 2·5 g; K_2HPO_4, 1·72 g; Na_2HPO_4 1·40 g; NaCl, 0·5 g; $MgSO_4 \cdot 7\ H_2O$, 0·45 g; $MnSO_4 \cdot H_2O$, 30 mg; $FeSO_4$, 12 mg; $CuSO_4$, 1 mg; $CoCO_3$, 1 mg; $ZnSO_4 \cdot 7\ H_2O$, 1 mg; KI, 0·3 mg.
[9] MacDonald-Bernier Co., Boston, Mass.
[10] P. TRINDER, Analyst *77*, 321 (1952).

Table

Group	No.	Sex M	Sex F	Weight (g)	Serum Cholesterol Mg %	(Range)
Six-Week Experiment						
Germ-free	10	2	8	540 ± 70	107 ± 25	(63–149)
Conventional	11	4	6	487 ± 33	120 ± 21	(86–168)
Eight-Week Experiment						
Germ-free	5	4	1	807 ± 48	144 ± 20	(108–170)
Conventional	5	3	2	579 ± 57	149 ± 17	(135–179)

suggest that in the absence of dietary cholesterol the intestinal flora of the chicken exert little or no effect on the serum cholesterol level. The effect of the intestinal flora in germ-free chickens fed cholesterol is under investigation.

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Résumé

On démontre qu'il n'y a pas de différence entre le cholestérol du sérum de poulets élevés dans des conditions aseptiques et ceux élevés dans des conditions normales et soumis à un régime sans cholestérol. Les poulets sans bactéries croissent mieux que les poulets normaux.

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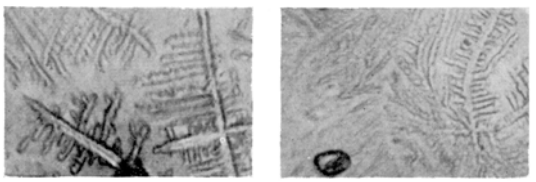
'Arborization' Phenomenon (AP) in Semen

Several years ago PAPANICOLAOU[1] observed a so-called 'arborization' phenomenon (AP) or palm (fern) leaf (PL) reaction in the cervical mucus secretion, which was spread on a slide and allowed to dry. The drying material, particularly if taken at the time of ovulation, crystallizes in an 'arborizatory' pattern. This interesting and clinically significant phenomenon stimulated a series of investigations *in vitro* as well as *in vivo*[1–9]. It has been proved that



a b

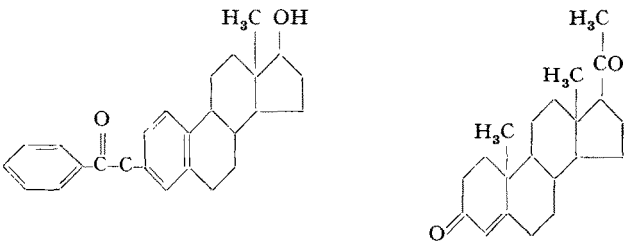
Fig. 1a–b.—Arborization phenomenon in semen. 450 ×.

any protein or carbohydrate substance may produce a PL or PL-like arborization phenomenon if it comes into the contact with electrolytes, such as NaCl, KCl, KBr etc.[2,4]. The occurrence or failure of the AP to appear, even though it still has 'PL-like' possibilities, may yet mask some factors which are beyond previous experimental conclusions.

After storage of semen of three different species (rabbit, bull, and human) one observed the PL phenomenon (Fig. 1a–b) which, in the series of experimentations, appeared more pronounced on the slide in the incubated (at 39°C) material than in the material stored at room temperature (RT) of all three species[9]. In most instances the PL reaction began to appear after about 5–6 days of incubation. The longer the material was incubated the more frequent and pronounced the PL appeared. The phenomenon, however, did not show essentially identical 'arborizatory' pattern in all cases. In most instances, the arborization consisted mainly of straight branches extending in a somewhat quadrangular shape, creating numerous figures, from which smaller side branches of different lenghts crossing and intercrossing distally with various-sized 'buds' of crystals frequently attached to the very ends. In other instances, however, the main lines showed curvations creating in some areas almost 'whorl-like' patterns with side branchings having somewhat indented leafy projections going toward the peripheries. Still in other cases, they appeared more or less pronounced if observed at a moderate microscopic magnification and with a partially closed diaphragm (see Fig. 1a–b).

It is probably true that incubation accelerated the degradation of certain protein products (peptides, tripeptides, polypeptides etc.) or even mono- or polysaccharides[6,10], which experimentally has been proved to have a definite relation with this phenomenon at certain concentrations (usually less than 4–6% a.s.o.). No AP or PL reaction, was observed in fresh seminal material, undiluted or diluted at room temperature (about 20°C). For the purpose of better microscopic identification the adding of dilute mixtures of the sodium salt of *p,p'*-dibenzyldiethyldiamino-*p''*-hydroxyphenylcarbinol trisulfonic acid anhydride ($C_{37}H_{34}O_{10}N_2S_3Na_2$) together with nigrosin or sodium salt of tetrabromofluorescein-nigrosin solutions[9], did not seem to interfere with that crystallization pattern.

On the basis of clinical observations, it was found that certain administered steroid substances[3–5, 11, 12] (e.g. Fig. 2a–b).



a Fig. 2a–b b

are closely related with the appearance or non-appearance of AP *in vivo* (PL structures in the material from patients obtained from 5–7th day to the 20–22nd day of cycle – ROLAND[5], CAMPOS DA PAZ[3]). Cervical as well as endometrial secretory activity ('glaire filante') of the uterus reflects in the administration (dosage) of those steroids[3,5]. There is a hope that further clinical as well as laboratory observations, in addition to the results obtained in veterinary field[8], will soon shed more light onto the nature of this interesting phenomenon.

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Worcester Foundation for Experimental Biology, Shrewsbury (Mass.), July 16, 1958.

[1] G. N. PAPANICOLAOU, Amer. J. Obstetr. Gynec. *51*, 316 (1946).
[2] E. RYDBERG, Acta Obstetr. Gynec. scand. *28*, 172 (1948).
[3] A. CAMPOS DA PAZ, Fert. Steril. *4*, 137 (1953).
[4] B. ZONDEK, *Recent Progress in Hormone Research*, vol. 10 (GREGORY PINCUS, Ed., Academic Press Inc., New York 1954), p. 395.
[5] M. ROLAND, Amer. J. Obstetr. Gynec. *63*, 83 (1952).
[6] W. T. POMMERENKE, Fert. Steril. *1*, 527 (1950).
[7] J. SÉGUY and H. SIMMONET, Gynécol. obstétr. *28*, 657 (1933).
[8] G. W. SCOTT BLAIR and F. A. GLOVER, Brit. vet. J. *113*, 417 (1957).
[9] A. F. GRINIUS, Unpublished results.
[10] T. MANN, *The Biochemistry of Semen* (Methuen and Co., London 1954).
[11] C. W. SHOPPEE, *Chemistry of the Steroids* (Butterworths scient. Publ. Ltd., London 1958).
[12] R. I. DORFMAN and J. B. HAMILTON, Endocrinology *25*, 33 (1939).