Purkinje cell demonstrates presence of myosin in myofibrils of the conduction bundle of beef heart. 2) Antibody to myosin whether prepared from skeletal muscle or heart tissue is capable of binding heart muscle antigen.

We wish to acknowledge our indebtedness to Dr. Koloman Laki for the opportunity for this research and many helpful suggestions.

1. Fink, H., Holtzer, H., Marshall, J. M., *J. Biophys. Biochem. Cytol.*, 1956, Suppl. 2, 175.

2. Klatzo, I., Horvath, B., Emmart, E. W., PROC. SOC. EXP. BIOL. AND MED. 1958, v97, 135.

3. Helander, E., *Acta physiol. scand.*, 1957, v41, Suppl. 141, Gothenburg.

4. Emmart, E. W., Cole, R. M., May, E. L., Longley, J. B., *J. Histochem. Cytochem.*, 1958, v6, 161.

5. Todd, T. W., *Special Cytology II.* E. V. Cowdry, Ed. Publ., P. B. Hoeber, 1932, 1175.

6. Schiebler, T. H., *Z. für Zellforsch.*, 1953, v39, 152.

7. ———, *ibid.*, 1955, v43, 243.

8. Truex, R. C., Curry, J. L., Smythe, M. R., *Anat. Rec.*, 1954, v11, 723.

9. Borg, T., *Acta neerld. morph. norm. et Path.*, 1941, v4, 97.

10. Muir, A. R., *J. Anat.* (London), 1957, v91, 251.

11. Caesar, R. G., Edwards, A., Ruska, H., *Z. für Zellforsch. und mikroskop. anat. Bd.*, 1958, v48, 698.

12. Moore, D. H., Ruska, H., *J. Biophys. Biochem. Cytol.*, 1957, v3, 261.

13. Fullmer, H. M., Lillie, R. D., *J. Histochem. and Cytochem.*, 1958, v6, 391.

14. Kamikozawa, Y., *Acta scholae med. univ. in Kioto*, 1923, v6, 133.

15. Edsall, J. T., *J. Biol. Chem.*, 1930, v89, 289.

16. Von Muralt, A. L., Edsall, J. T., *ibid.*, 1930, v89, 315.

17. Weber, H. H., *Ergebn. Physiol. exp. Pharmakol.*, 1934, v306, 109.

18. Kesztyüs, L., Nikodemusz, S., Szilagyi, T., *Nature*, 1949, v163, 136.

19. Ebert, J. D., *J. Zool.*, 1953, v39, 333.

20. Szent-Gyorgyi, A., *Chemical physiology of contraction in body and heart muscle.* Academic Press, 1953.

21. Weber, H. H., Portzehl, H., *Advanc. Protein Chem.*, 1952, v7, 162.

22. Laki, K., *J. Cell. Comp. Physiol.*, 1957, v49, Suppl. 1, 249.

23. Holtzer, H., Abbott, J., *Anat. Rec.*, 1958, v131, 417.

Received June 10, 1959. P.S.E.B.M., 1959, v101.

The Fetal Lung, A Source of Amniotic Fluid. (25115)

IVO SETNIKAR, EMILIO AGOSTONI AND ALESSANDRO TAGLIETTI
(Introduced by J. S. Nicholas)
Instituto di Fisiologia Umana, University of Milan, Italy

The physico-chemical features of amniotic fluid in last third of pregnancy are very similar to those found in interstitial fluid(1), particularly in animals in which the allantoic sac is still present during this period, *e.g.*, goats and sheep(2). Amniotic fluid could therefore be produced by an ultrafiltration process. This production requires an organ with large surface supplied by many capillaries, in which hydrostatic pressure is higher than colloid-osmotic pressure of plasma proteins (14-20 mm Hg in goat fetus(3)). Two organs present such properties: kidneys and lungs. Hydrostatic pressure in pulmonary capillaries should be higher than colloidosmotic pressure, as pressure in pulmonary artery is relatively high, due to the nature of fetal circulation. Pressure in the pulmonary artery is higher than that found in the aorta and peripheral arteries, where pressure reaches values of about 70-80 mm Hg(4) at end of pregnancy. The kidney cannot be the only source of amniotic fluid inasmuch as amniotic fluid is present also when the fetal urethra is not patent and the urine must pass through the urachus into the allantoic sac, as in goats and sheep, to 100-120 days pregnancy, *i.e.*, to its last third. This research investigated the possible production of fluid by the lungs of fetus and to measure its rate of flow.

Methods. Experiments were performed on goats and guinea pigs during last third of pregnancy. The goats lightly anesthetized with chloralose (70 mg/g intravenously) were

TABLE I. Fluid Production from Lung of Goat Fetuses and Physico-Chemical Features of Bronchial and Amniotic Fluid.

Fetal age, days	Fetal wt, g	Period of fluid production, hr	Vol of fluid production, ml	Flow, ml/kg/hr	Osmotic pressure, mOsmol		Protein, % content	
					Bronchial fluid	Amniotic fluid	Bronchial fluid	Amniotic fluid
158	755	2	.11	.073	302	304	.1	.2
117	1070	2	.59	.280	304	289	.2	.2
118	1460	*			310	293	.2	.2
105	900	*			295	300	.2	

* Flow of liquid from the trachea was large in first 10 min. (to 20 ml), and then ceased.

placed, in supine position, in a bath of Ringer solution at 38°C. After opening the abdomen the position of the fetus was found, and the head delivered. Keeping the head under Ringer solution, the trachea was cannulated. These operations were performed under water to prevent air from entering into airways of fetus. The tracheal cannula was connected to glass tubing by a polyethylene catheter filled with saline. The glass tubing was positioned at the same level of liquid in which the fetus was placed, to counterbalance the hydrostatic pressure of the saline solution above the fetus. After replacing the head of the fetus, the uterus was sutured. Loss of amniotic fluid during the operation was reduced to a small amount. At end of experiment the osmotic pressure of amniotic fluid and of the liquid contained in the bronchi, was measured by freezing point technic, and protein content was determined by a refractometric method. Guinea pigs were anesthetized with sodium pentobarbital (40 mg/kg intraperitoneally). A small incision was made in wall of uterus above the fetal trachea. The trachea was cannulated and connected to a glass tube without delivering the head of the fetus. Uterus and abdomen were then sutured. All these operations were performed under saline solution. Displacement of liquid in the respiratory apparatus could be measured by movements of the meniscus in the glass tubing.

Results. Four experiments were performed on goats.* In 2 experiments fluid came out of the trachea continuously for 2 hours. In the other 2, the flow stopped after about 10 minutes. During this time a large amount of fluid was produced (Table I). Liquid coming out of trachea was clear, colorless and the osmotic pressure and protein content was similar to those found in amniotic fluid.

Ten experiments were performed on the guinea pig, and there was no evidence of fluid entering the trachea. The flow of liquid out of the trachea during first 5 minutes was disregarded as probably due to a change in lung volume.

In 4 experiments the flow of liquid from the trachea was negligible (0 to 0.05 ml) and hence has not been reported. In the remaining 6 experiments the liquid came out for 30 to 240 minutes with a flow of 1.3 to 6.7 ml/kg/h.

Discussion. In some experiments on the guinea pig the amount of liquid that flowed slowly and continuously from the trachea was larger than total amount of fluid that could be contained in lungs at beginning of experiment. This finding demonstrates that the fetal lung produces fluid which is similar to amniotic fluid.

These experimental results support the hypothesis of Jost and Policard(5). These authors observed an increase of lung volume of rabbit fetus after tying the trachea, and they thought that amniotic fluid could be produced also by the lungs.

This research can also explain the observa-

TABLE II. Fluid Production from Lung of Guinea Pig Fetuses.

Fetal wt, g	Period of fluid production, hr	Vol of fluid produced	Flow, ml/kg/hr
100	4.0	1.10	2.8
94	2.5	1.00	4.3
90	1.0	.12	1.3
86	1.0	.12	1.4
120	1.25	1.00	6.7
100	.5	.15	3.0

* These experiments were performed in the Dept. of Physiology of Yale Medical School.

tion of Reynolds(6), that 30 ml of liquid flowed from the nasopharyngeal oral cavity of a goat fetus in a 2 hour period, and confirms the observation and the hypothesis of Dawes (7).

Inasmuch as no liquid entered the trachea during experiments, the hypothesis that amniotic fluid is absorbed by the lung seems improbable. This hypothesis was proposed by Davis and Potter(8), on the basis that radioopaque material injected into the amniotic sac was found in the bronchi, but this result can be explained by respiratory movements of the fetus. If amniotic fluid were absorbed in the lung the corpuscles that normally are present in the amniotic fluid would be collected in the lung causing impairment of respiratory function at birth.

The volume of liquid coming out in these experiments represents likely minimal values. If the hypothesis of an ultrafiltration is correct a decrease of hydrostatic pressure in the capillaries would be sufficient to decrease or discontinue the production of fluid. It seems quite probable that such a decrease takes place as a consequence of anesthesia and of surgical shock.

The lung seems to be a source of amniotic fluid, and of particular importance in those animals in which the urethra becomes patent only late in fetal life. In animal in which the urethra becomes patent earlier (*e.g.* man and guinea pig) the kidneys also contribute to production of amniotic fluid. This organ produces a large amount of hypotonic fluid in the last third of fetal life, without waste products found in urine of extrauterine life. The amount of fluid produced by kidneys of the fetus is 6-10 ml/kg/h in goats(9), 0.1-1 ml/kg/h in guinea pigs(10), and about 1 ml/kg/h in rats(11).

Both lungs and kidneys in the guinea pig can therefore produce amniotic fluid at a rate of 1-10 ml/kg/h. This rate of production is about 10-100 times less than the rate of transfer of water to the amniotic fluid found in the guinea pig by Flexner and Gellhorn(12). However, as shown by Paul, *et al.*(13), these results are mainly due to interdiffusion phenomena of water through the permeable barriers that enclose the amniotic cavity and are therefore not the expression of a net transfer or a production of water. This production of fluid is likely small, inasmuch as there is not a continued and large flow of liquid after rupture of the amniotic sac.

It is therefore important to distinguish between interdiffusion and net production of fluid. Interdiffusion is a general phenomenon (less intense in the amniotic sac than in other parts of the organism(14)), resulting from thermic movement of molecules and taking place through every water-permeable membrane. The net production of fluid is caused by ultrafiltration or secretion, and requires energy expenditure and specialized organs. Only the net production of liquid can give rise to a collection of fluid in the organism, and the amniotic fluid is an example of this collection.

Summary. Fluid production by fetal lung of guinea pigs and goats has been demonstrated through cannulation of the fetal trachea in the last part of the pregnancy. This fluid is probably produced by an ultrafiltration process because its physico-chemical features in the last third of pregnancy are very similar to those of the interstitial fluid. It is suggested that the fetal lung is an important source of amniotic fluid during this period of fetal life.

We are grateful to Dr. Donald H. Barron for continued interest, encouragement and suggestions throughout this study.

1. Hanon, F., Coquoin-Carnot, N., Pignard, P., *Le liquide amniotique,* Paris, Masson, 1955.

2. Alexander, D. P., Nixon, D. A., Widdas, W. F., Wohlzegen, F. X., *J. Physiol.,* 1958, v120, 1.

3. Meschia, G., *Am. J. Physiol.,* 1955, v181, 1.

4. Barcroft, J., *"Research on Prenatal Life."* Oxford, Blackwell, 1946.

5. Jost, A., Policard, A., *Arch. Anat. Microsc.,* 1948, v37, 323.

6. Reynolds, S. R. M., *Nature,* 1953, v172, 307.

7. Dawes, G. S., *Cold Spring Harb. Symp. Quant. Biol.,* 1954, v19, 164.

8. Davis, M. E., Potter, E. L., *J.A.M.A.,* 1946, v131, 1194.

9. Alexander, D. P., Nixon, D. A., Widdas, W. F., Wohlzogen, F. X., *J. Physiol.,* 1958, v140, 14.

10. Boyland, J. W., Colburn, E. P., McCance, R. A., *ibid.,* 1958, v141, 323.

11. Wells, J., *Anat. Rec.,* 1946, v94, 504.

12. Flexner, L. B., Gellhcrn, A., *Am. J. Physiol.*, 1942, v136, 757.
13. Paul, W. M., Erms, T., Reynolds, S. R. M., Chinard, F. P., *J. Clin. Invest.*, 1956, v35, 634.
14. Pinson, E. A., *Physiol. Rev.*, 1952, v32, 123.

Received June 15, 1959. P.S.E.B.M., 1959, v101.

Biological Activities of Aggregated Gamma Globulin I. Skin Reactive and Complement-Fixing Properties of Heat Denatured Gamma Globulin.* (25116)

TERUKO ISHIZAKA AND KIMISHIGE ISHIZAKA†
(Introduced by M. M. Mayer)
Depts. of Microbiology and Medicine, Johns Hopkins School of Medicine, Baltimore, Md.

In earlier publications on skin reactivity and complement (C′) fixing potency of soluble antigen-antibody complexes, it was shown that these biological activities are dependent on 2 properties of the complexes, *i.e.*, their composition with respect to antigen/antibody ratio and the species from which the antibody was derived(1,2). When complexes were composed of immune reactants in the molar ratio Ag_2Ab or when the antigen was a simple hapten, the biological activities of skin reactivity and C′-fixation were lacking. Moreover, it was observed that formation of soluble complexes with biological activities was accompanied by increase in levorotation (3). A mechanism for the observed changes in optical rotation was suggested, which postulated that the increase in levorotation might be due to interaction between antibody molecules brought into apposition by antigen. The possibility was also considered that this antibody-antibody interaction might result in induction of skin reactivity and fixation of C′ (3). These considerations were restricted to those complexes containing human or rabbit antibody which manifested the biological activities. It was therefore anticipated that the interaction of human or rabbit antibody (gamma globulin) molecules, induced by reagents other than specific antigen, might also be accompanied by a development of skin reactive and C′-fixing properties. Since it has been shown that the molecular configuration of gamma globulin changes on heating with formation of aggregates, this denatured protein was used to test this possibility. The experiments described below indicate that human gamma globulin aggregated by heat can provoke skin reactions in normal guinea pigs and can also inactivate C′. Aggregated bovine gamma globulin exhibits neither of these properties.

Materials and methods. Gamma globulin. Two preparations of human gamma globulin (HGG) were used, a pooled commercial human Fr. II (Squibb) and a preparation obtained through the courtesy of Dr. W. L. Hughes.‡ The bovine gamma globulin (BGG) was Fr. II (Armour & Co.). In free boundary electrophoresis in barbital buffer (pH 8.6, $\mu = 0.1$), the purified HGG (Hughes) showed a single sharp peak. The BGG contained approximately 5% of a component with mobility of -2.6×10^{-5} cm^2 volt^{-1} sec^{-1}. The tests for skin reactivity of gamma globulin were carried out in guinea pigs in the same manner previously described for testing activity of soluble antigen-antibody complexes(4). The gamma globulin preparations were injected in

* These experiments were performed while the authors were in residence in Depts. of Microbiology and Medicine, Johns Hopkins School of Medicine and Public Health. This work was done under auspices of Commission on Cutaneous Diseases of Armed Forces Epidemiological Board, and supported by Office of Surgeon General, Department of Army, and National Science Fn.

† Fulbright Fellow; Permanent address: National Inst. of Health, Tokyo.

‡ The authors are grateful to Dr. Asger Langlykke, Director of Squibb Inst. for Medical Research and Dr. Walter L. Hughes of Brookhaven National Labs. for these preparations.