the species present. Kessel[2] and Chiang[3] have both reported that amebae-free rats may be selected from colonies in which amebic infections are present. It is possible to find older animals that appear to be entirely free from amebae but, when these animals breed, the young are invariably infected with amebae at the time they are weaned, even though they have not been in contact with any rats except the apparently amebae-free mothers. In view of Kessel's statement that "the most advantageous time to choose rats for feeding experiments is shortly after they are weaned or about the age of two months. Among rats of this age will be found the greatest number free from amebic infections . . ." it seems that his method for detecting amebic infections is not very effective with young rats. Out of two hundred examinations on young rats between four and twelve weeks of age that had been left with infected mothers until they were weaned, not one has been found free from amebae.

In the course of observations on the intestinal contents of rats between ten and thirty days old, it was noted that protozoa did not appear in the intestine of young rats until the rats were twenty to twenty-five days old, and had begun to eat solid foot. It seemed, therefore, that if it were possible to separate young animals from the mothers sixteen to eighteen days after birth, before they became infected (that is, before they began eating solid food) and raise them without further contact with infected rats, that they should remain free from intestinal protozoa.

Accordingly, fifteen young rats, seventeen days old, were separated from the mothers and placed in a clean cage on shavings. During the first two days they were fed whole milk powder dissolved in warm water, the milk being prepared and given fresh three times a day. At first, only a few of the rats would drink from shallow dishes of milk placed in the cage. The others had to be fed by means of a pipette, but after being fed in this way several times they, too, began drinking. On the third day they were started on the stock ration which consists of whole wheat flour 60, whole milk powder 15, unpurified casein 15, butter 5, calcium carbonate 2, ferric citrate 2 and sodium chloride 1, and given water and green vegetables.

Four of these rats died during the first three days, but, from then on, the other eleven grew rapidly. When they were four weeks old, five of them were killed and the contents of the large and small intestine and cecum were examined microscopically for protozoa. The remaining six were similarly examined when they were eight weeks old, and all of them were apparently uninfected. This first group of rats came from two litters, seven in one and eight in the other, and it seemed that better results might have been obtained had the litters been smaller. Hence all the other rats employed came from litters that had been reduced to four animals each. These young were separated from the mothers at sixteen days of age, and none of them died.

The second group, raised in the manner described above, consisted of eight animals. They were kept until they were eight weeks old, then killed and examined, and were negative for intestinal protozoa. The third group also consisted of eight rats, five females and three males. When these animals were twelve weeks old 0.2 cc of material was removed, by surgical operation,[4] from the cecum of each and examined microscopically for protozoa, but apparently these rats were also uninfected. This examination has been found to be entirely sufficient to determine the presence of protozoa that live in the cecum of rats, but does not allow an examination of the small intestine for *Giardia*. Hence these rats were allowed to breed, and half the young from the first litters were killed and examined when they were weaned, but were found to be uninfected. It seems safe to say, then, that a breeding stock of "protozoa-free" rats may be established by this method.

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SPECIAL ARTICLES

ACCUMULATION OF GAS IN CLOSED COLLODION SACS IMMERSED IN FLOWING TAP WATER

COLLODION sacs, 16 mm in diameter and about 60 mm long, filled with water or with aqueous solutions of various salts or gases and firmly closed without inclusion of free gas bubbles, when placed in slowly flowing water direct from the Baltimore City water system in April and May of this year, became completely filled with gas in from six to seven days; then during the next few days the amount of gas continued to increase until the walls became quite tense from the evident development of a pressure within the sacs markedly greater than atmospheric. Professor W.

[2] J. F. Kessel, 1923, "Methods of Obtaining Amebae-free Rats for Experimental Infection with Intestinal Amebae." Univ. Calif. Pub. Zool., 20: 401–408.

[3] S. F. Chiang, 1925, "Study of Parasitic Amebae by Experimental Cross-infection of Laboratory Animals," *Nat. Med. Journ. China*, 11: 440–482.

[4] H. L. Ratcliffe, 1928, "The Numbers of Trichomonads in Rats on Diets of Different Protein Content in Relation to the pH and Bacteria in the Cecum," *Amer. Journ. Hyg.*, 8: 910–934.

A. Patrick, of the chemistry department of this university, kindly analyzed a sample of this gas for me and reported that oxygen, carbon dioxide and nitrogen (including other inert gases) were present in normal atmospheric proportions. No bubbles appeared in control sacs placed in flowing tap water which had been submitted in five-gallon bottles to the action of the partial vacuum created by a faucet filter-pump until only occasional bubbles were given off.

The fact that gases in solution in water pass through the walls of such sacs in this positive manner is not recorded, so far as I have been able to ascertain, and certainly is not well known. It seems probable that this is only a particular case of an unrecognized phenomenon common to the behavior of all gases dissolved in liquids and in contact with semi-permeable membranes, and is therefore of interest in several fields of science. For these reasons, it seems advisable to give a more detailed account of my experiments than would otherwise be justified.

Serious distortions of the membranous labyrinth are very common in temporal bone preparations. Since the bone present prevents observation of the effects of each stage of preparation upon this thin-walled closed system mostly surrounded by fluid-containing spaces, closed collodion sacs and glass jars have been utilized in an effort to determine the possibly injurious steps by this rather crude imitation of actual conditions. Collodion sacs of the so-called "moist" type were prepared in the usual way, using test-tubes and an 8 per cent. solution of Parlodion dissolved in equal parts of absolute alcohol and ether. Many such sacs, completely filled with one fluid and with the open end closed by a solid rubber stopper secured by several turns of stretched rubber cord, have been placed in jars of the next fluid used in several methods of preparation. Collodion sacs can not, of course, be used for the study of the effects of the higher alcohols and celloidin nor of fixation as such, but the method has proved very useful for direct observations at other stages.

It was in the course of thus attempting to imitate the standard histologic procedure of washing tissues in running tap water to remove certain fixing fluids or acids or sodium sulphate after acid that the unexpected phenomenon here recorded was stumbled upon. Sacs were filled with each of these several solutions and immersed in jars of running tap water. Whenever this was done, in addition to the volume changes due to osmosis and the diffusions that were to be expected from the nature of the particular fluid, a small gas bubble appeared inside of the sac in the course of a few hours and by the end of two days (a common time for washing large blocks like human temporal bones) was of considerable size. At first I attributed this to the simple release of dissolved air from the water, possibly supersaturated with air while under pressure in the piping system, which had passed into the sac in accordance with well-known laws of osmosis before a complete balance had been reached after release from the faucet. I had suspected this as a possible source of small bubbles in the labyrinth. That this is not a sufficient explanation was soon obvious when some sacs were left in running tap water for a longer time. The gas bubbles continued to enlarge until they completely filled the sacs, and it was noted that the larger the gas bubble at any given time the greater was the amount of gas added during the next equal time interval. Sacs 16 mm in diameter and about 60 mm long became completely filled with gas in from six to seven days, and it was very common for the last half of the filling to take place during the last twenty-four to thirty-six hours. This occurred with sacs filled with any one of the several aqueous solutions and placed in a jar through which was flowing a slow stream of water direct from taps of the Baltimore City water supply during April and May of this year.[1] After visible fluid had completely disappeared and the sacs were filled with gas, if left in the running water the walls became distinctly tense, indicating that the contained gas had increased in amount until it was under pressure markedly greater than atmospheric. The walls of several sacs even gave way.

Some sacs were then filled with distilled water and others with tap water and placed in running tap water in the same way to see if the particular solutions used in the original fillings were in any way responsible, and also whether a preliminary osmotic action due to dissolved salts was an essential factor. So far as concerns filling with gas and developing internal pressure after filling these all behaved in exactly the manner already described. Several repetitions gave identical results. Therefore the presence of dissolved salts does not seem to be an important factor. Further, the rate of increase of the size of the gas bubble during the period of forcing out the contained fluid is evidently not correlated with the amount of water possibly diffusing into the sac and then releasing its surplus gas, but would seem to be associated in some way with the amount of surface of the sac wall in contact with the contained gas.

As controls, tap water which had been partially "degassed" by the action of a faucet filter-pump until bubbles had almost ceased to be given off (thirty minutes to two hours, according to the water pres-

[1] The time of year and the location are specifically indicated because I have some reason to believe that both are factors of importance. At present (late June) it takes longer for similar sacs to fill, and in early June no gas appeared when this was tried in Wilmington.

sure) was siphoned through jars in which were placed similar sacs filled with the same fluid. No bubbles whatsoever appeared in any of the sacs, and when these same sacs with their contents were transferred after several days to untreated tap water, in order to control the possibility that these particular sacs were impermeable, the appearance of the first bubbles was much delayed (from three to five days), but after the bubbles began to form the rate of increase was as before. When sacs which had become partially or completely filled with gas from the ordinary tap water were transferred to jars of running "degassed" tap water (faucet filter-pump treatment) the bubbles gradually disappeared. In three days a sac which had been quite tense with gas became almost completely collapsed. Upon returning it to ordinary tap water it again filled with gas and became tense.

The actual amount of pressure developed by the accumulated gas has been measured in only one sac, and this was done during June, at which time other sacs did not fill as quickly or seem as tense as during April and May, when I believe the values obtained would have been somewhat greater. The sac, filled with tap water and surrounded by running tap water in a suitable container, was connected through a perforated stopper to a water manometer with a leveling bulb arrangement for adjustment of the column height without disturbance of the gas pressure to be measured. In ten days (middle of June) the sac had become completely filled with gas; during the next four days the pressure gradually rose until it reached a maximum that supported 49½ inches of water (a 5-mm diameter tube was used to minimize the surface tension factor). For several days then there was a fluctuation of from two to three inches, apparently correlated with temperature variations in the issuing tap water. Whenever many other faucets on the line were open so that the water was cooler there occurred a decrease of considerably more than can be accounted for by the temperature change of the gas itself. It seems reasonable to assume that the pressure was correlated also with the degree of saturation of the water with gas when freshly released from the piping system at different temperatures. After making such readings for several days tap water which had been submitted to the action of a faucet filter-pump for two hours (and which was warmer than any of the direct tap flow) was run through the container around this sac. The pressure of the gas in the sac fell to zero in ten hours (six gallons of water were used in this time). Then the direct tap connection was again made and in three days the pressure increased to its previous level, thus proving that the fall in pressure was not due to leakage developing coincident with the use of the "degassed" water.

Rigid control of each possible factor, including the study of other membranes, fluids and gases, is needed to determine the laws governing this phenomenon and to work out the true explanation of it. I am not in a position to conduct or to direct such a study, and therefore can only express the hope that physical chemists or others will carry on. When worked out these purely physicochemical laws concerning inert membranes and gases in solution will probably be found of much assistance in understanding some of the phenomena of living matter, such as the gaseous exchanges in respiration and the so-called secretions of gas in various forms.

The significance of these observations as regards distortions of the membranous labyrinth is obvious, and will be dealt with in detail elsewhere; it is equally obvious as regards all the other fields of histologic work in which cavities and delicate tissues are associated and where gas bubbles frequently cause serious damage (embryology, for instance). For the benefit of others who may be troubled with gas bubbles in their tissues a few applications of these observations will be briefly indicated. It is practicable to reduce by filter-pump evacuation the dissolved gas content not only of the water used, but also of many fixing fluids and of the lower strengths of alcohols, and from my own experience to date I am quite certain that by means of this simple procedure (carried out with each fluid just before use, not after the specimen is in the fluid) it is possible not only to prevent the formation of internal bubbles but even to withdraw bubbles that are present without pricking into or cutting valuable specimens. The rate of reabsorption from the atmosphere is sufficiently slow (unless the container is violently agitated) that it is not necessary to cover the surface with oil if a reasonably deep layer of fluid is used and changed daily and the specimen placed well below the upper surface. Even freshly distilled water contains so much air, from absorption during the condensed film stage, that it does not prevent formation of some bubbles and has little or no tendency to absorb bubbles already present unless it has been freshly "degassed." Formalin solutions or fluids containing formalin give off much formaldehyde when acted upon by a filter-pump, but bubble formation is diminished if the water (or the Zenker's or Müller's fluids, for instance) is "degassed" just before adding the formalin required.

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DOES REGENERATION FOLLOW COMPLETE OVARIOTOMY IN THE ALBINO RAT?

SINCE the time when August Weismann first made a clear distinction between the soma and germ-plasm