also satisfactory. Figs. 2A and 2B illustrate the responses of two different geophysical galvanometers (Miller) to a 200 millivolt signal applied to the grids of the output tubes. The current change is approximately 15 microamperes. With a single pentode (6C6) RC-coupled to the output tubes to provide amplification (Fig. 1A), a 500 microvolt signal gives a good deflection, as shown in the records (Fig. 2C) obtained in response to the "on" and "off" of a 500 microvolt square wave. The voltage amplification, as measured with the cathode ray oscillograph, is about 100. The time constant of this amplifier is about 1.5 seconds, but this figure could be increased by the use of a double RC coupling[7]; or even direct coupling could be employed. The ability of this amplifier and the Miller galvanometers to record such physiological processes as the electrocardiogram and finger pulses is shown in Fig. 2 (D, E, F). The finger pulse records are similar to those taken with the cathode ray oscillograph.[8]

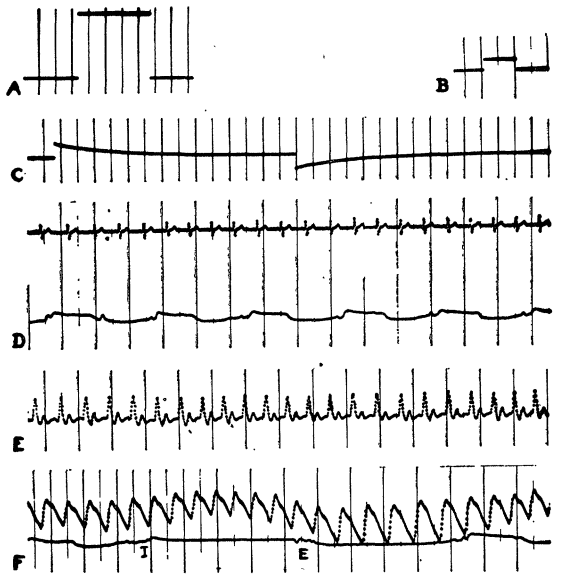

FIG. 2. A and B. A 200 millivolt square wave applied to the output tubes of amplifier (1A) and recorded by two different Miller galvanometers. C. The "on" and "off" of a square wave (500 microvolts) applied through amplifier (1A). D. The electrocardiogram (lead 1) and respiration recorded simultaneously. A thermocouple mounted in a nose piece was used to record respiration, the thermocouple operating the galvanometer directly. Upward deflections represent inspiration. E. Pulse in the right index finger recorded by the use of the hot wire-thermocouple method.[6]. F. Pulse in the right finger recorded by means of a phototube and showing the effects of a prolonged inspiration (beginning at I) and the effect of a prolonged expiration (beginning at E). Time throughout is in half seconds.

The special advantage of these geophysical galvanometers is that they combine, as few other galvanometers do, the qualities of sturdiness, sensitivity and good frequency response. Many pick-up units such as strain gages, thermocouples, thermopiles, photocells, etc., can be used either coupled directly to the galvanometer or through a simple matching stage. This makes unnecessary the need of high-gain amplifiers, which, especially in the low frequency (subaudio) range, involve special design problems or special precautions in order to achieve a reasonable degree of stability. For some types of physiological recording it may be necessary to utilize a balanced or differential input stage. Quite adequate is Matthews's[9] circuit employing two tubes with a common cathode resistor and a negative cathode voltage. A slightly modified arrangement (Fig. 1B) is very satisfactory and possesses a high differential action. When added to the amplifier (Fig. 1A) the total voltage amplification is about 1,000, and 50 microvolts gives a good deflection.

It would seem that the use of these oscillographs in physiology would simplify many instrumental problems and give to the physiologist and biologist a convenient and useful tool for simultaneous multiple recording of many physiological changes in either one or several organisms.

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DISCUSSION

THE AMINO ACID COMPOSITION OF PROTEINS

IN a recently published monograph on methods for the amino acid analysis of proteins,[1] the practice is advocated of computing the results of the determinations upon the uniform basis of a hypothetical substance that contains 16 per cent. of nitrogen. Comprehensive tables of data are presented, in all of which the original figures from the literature have been recalculated. The authors state that this is done

[1] R. J. Block and D. Bolling, "The Amino Acid Composition of Proteins and Foods." Springfield, Illinois. 1945.

[7] F. E. Terman, *Radio Engineers' Handbook*, 1st edition, p. 374. McGraw-Hill Book Co., Inc., New York, 1943.

[8] F. Crescitelli and E. Gardner, *Jour. Lab. Clin. Med.*, 30: 63, 1945.

[9] B. H. C. Matthews, *Jour. Physiol.*, 93: 25P, 1938.

[10] Now with the Department of Anatomy, College of Medicine, Wayne University, Detroit.

"in the interest of uniformity and to facilitate comparison," but they fail to explain why they think, for example, it is easier to compare the results of the analysis of casein, a substance that contains close to 15.7 per cent. of nitrogen, with similar results for edestin, which contains about 18.7 per cent., after both groups of data have been recalculated in this way. Actually, this procedure gratuitously introduces an element of inaccuracy. It is the equivalent of suggesting that, prior to analysis, protein preparations of nitrogen content greater than 16 per cent. should be diluted with sufficient non-nitrogenous material to reduce the average nitrogen content to this level, while protein preparations of lower nitrogen content should be in some way fortified with nitrogen. The values for the percentage yields of amino acids determined by analysis are thus distorted, this distortion being the greater the more the actual nitrogen content of the preparation deviates from the assumed 16 per cent.

Proteins are regarded by most investigators as comprising a fairly clearly defined category of organic substances, although their properties and their composition with respect to constituent amino acid residues may vary within extremely wide limits. The well-known differences in their nutritive effect in the animal body are made neither more nor less striking if the composition is expressed in the manner described, nor is the basis of any of their properties save nitrogen content thereby equalized. It has never been suggested that the consideration of any other broad group of organic substances would be facilitated if the composition were expressed upon an analogous artificial basis. Why, then, should the proteins receive so unusual a treatment?

It is possible that the suggestion has its origin in the convention which has long been employed, in the absence of information upon the actual nitrogen content of the proteins in a solution or tissue, that an approximation to the weight of the protein present may be secured by multiplying the nitrogen content of the solution or tissue by the factor 6.25. This treatment assumes that the proteins contain, within the limits of accuracy desired, 16 per cent. of nitrogen. Aside from the protamines and certain seed globulins, which are especially rich in arginine, there are few proteins that contain more than 18 per cent. of nitrogen. Also, with the exception of certain complex materials, many of which appear to be associations or compounds of protein with lipides or with carbohydrates, there are few protein preparations that, in a satisfactorily purified condition, contain much less than 15 per cent. of nitrogen. Accordingly, the use of the conventional factor would give a result about 11 per cent. too high in the case of a protein that actually contained 18 per cent. of nitrogen, and about 6.5 per cent. too low if it contained 15 per cent. For some purposes, such a range of uncertainty is not objectionable, and many proteins of importance in animal biochemistry do not differ widely in nitrogen content from the 16 per cent. that is assumed. Nevertheless, the magnitude of the error may become serious with such substances as gelatin or silk fibroin and especially when proteins of vegetable origin are under consideration. This last point was carefully emphasized by Ritthausen[2] as long ago as 1872 and was discussed thoroughly by Jones[3] in 1931.

In 1901, Kossel[4] delivered a lecture before the German Chemical Society, which he concluded with the following statement. "It has been customary to think of protein as a substance of definite fixed properties, a sort of 'ideal protein' much as Goethe thought of an *'Urpflanze'* or 'ideal plant.' Those protein substances that did not correspond to this ideal have been supposed to be defective and have been placed in a lower group such as the albuminoids. This point of view can no longer be maintained. It is a necessity in present-day science to regard each organic substance as a member of an evolving series, a necessity that has its most striking expression in the direction that has been taken by phylogenetic and ontogenetic research. We must not, therefore, consider one complicated protein molecule to be representative of all, but must seek to find a system of proteins which, progressing from the simplest to the most complex, reveals to us the innermost character of these many-sided substances."

It would seem that the recalculation of the amino acid composition of all proteins, whatever their nitrogen content, upon the arbitrary basis that has been suggested is a return to the point of view objected to by Kossel and represents a step that, as long as forty-five years ago, he would have regarded as a backward one. The data of the protein analytical chemist are to-day finding application in many directions. They are used for the characterization and differentiation of proteins, for the study of their molecular composition, in the interpretation of their chemical and physical properties, in studies of metabolism and of protein nutrition, and they will doubtless find ultimate use in the explanation of the enzymological and immunological properties of proteins

[11] Now with the Medical Research Laboratory, Edgewood Arsenal, Edgewood, Maryland.

[2] H. Ritthausen, "Die Eiweisskörper der Getreidearten, Hülsenfrüchte und Ölsamen," p. 236. Bonn: Max Cohen u. Sohn. 1872.

[3] D. B. Jones, U. S. Dept. Agric. Circular 183, 1931.

[4] A. Kossel, *Ber. chem. Ges.*, 34: 3214, 1901.

and in the development of a comprehensive theory of protein structure. These are fields of great activity in biochemistry, and in all of them the demand is for accuracy and more accuracy.

The employment of a method of calculation which introduces needless error without any compensating advantage is strongly to be condemned. This is not the time for a backward step, for a relaxation of the standards of accuracy or for the introduction of a new complication into an already sufficiently complex situation.

Attention is drawn to this matter because several papers have recently appeared in which the use of the proposed method of calculation has apparently escaped the vigilance of the referees, and more are to be anticipated.

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RELATIONSHIPS OF LYMPHOCYTES AND CANCER[1]

FINDING a direct correlation between the amount of lymphoid tissue in the intestine and the incidence of spontaneous mammary tumors in several pure strains of mice[2] suggested the idea that the lymphocyte might contribute to the growth of tumors or possibly even to instigate them. Lymphocytes have been considered in many studies on cancer, but practically all the experiments have been designed from the viewpoint that this cell and the lymphoid tissue has a part in immunity to tumor transplants. The conclusions obtained have not been consistent, one group of investigators maintaining that the lymphocyte is a factor to immunity; the other, that this cell has no part.

The approach used here is based upon histological association between the presence of lymphocytes and tumors and also upon correspondence in the growth and incidence of tumors and conditions that result in an increase or decrease in the number of lymphocytes within a tissue or throughout the body as a whole.

Mammary tumors. Records of metastases of mammary carcinoma in women and data obtained from animal experiments indicate a connection between mammary tumors and the presence of lymphocytes. Metastases of mammary carcinoma occur most frequently in lymph nodes, organs normally composed chiefly of lymphocytes. In addition, lymphocytes are present in metastases to the liver,[3] in neoplastic foci developed from burst lymphatics[3] and between the elastic connective tissue and the epithelium containing neoplastic cells in Paget's disease of the nipple.[4]

Animal experiments indicate several histological associations between lymphocytes and mammary tumors. The mammary tumor inciter in mice has been found in the milk, blood, spleen, thymus, lactating mammary tissue and breast tumors of certain strains high in their incidence of spontaneous mammary tumors.[5, 6] No cell is as numerous in all these fluids and organs as is the lymphocyte; it is a dominant histological element in the spleen and thymus gland. Furthermore, lymphocytes occur in the milk of many animals and have also been identified in the stomach contents of nursing mice. After centrifuging milk diluted with saline, the lymphocytes occur chiefly in the sediment, very few remaining in the supernatant fluid. The milk factor in mice has been found to be concentrated in the sediment.[7]

Breeding in pure strains of mice shows another connection between the incidence of spontaneous mammary tumors and lymphocytes. For example, strain dba has only 51 per cent. tumor incidence in virgins, but 85 per cent. in the breeding females; strain A shows a more marked difference by having less than 5 per cent. in virgins and 84 per cent. in breeding females.[8] One prominent histological change in the mammary gland during pregnancy and lactation is the presence of quantities of lymphocytes. There are masses of lymphocytes in the mammary gland of mice belonging to line dba during various stages of pregnancy and lactation.[9]

Lymphocytosis. The term "lymphocytosis" as used here will apply not only to an increase in the circulating lymphocytes, but also to an increase in the number of lymphocytes within an organ or tissue.

Data exist to show that one inciter of tumors is irritation, whether it be caused by chemicals, physical factors, radiation or possibly by certain parasites and viruses. Regardless of its source, irritation produces histological changes involving the attraction of the white blood cells. Lymphocytes are present at neoplastic foci caused by irritation.

The increase in blood lymphocytes that occurs in some unbalances of the endocrines and in some in-

[1] The author is a recipient of a Finney-Howell Research Fellowship.

[2] M. A. Kelsall. Paper in press.

[3] W. S. Handley, "Cancer of the Breast and Its Treatment." New York: Hoeber. 1922.

[4] G. L. Cheatle and M. Cutler, "Tumors of the Breast." Philadelphia: Lippincott.

[5] J. J. Bittner, *Trans. and Stud., College of Physicians of Philadelphia*, 4 ser., 9: 129–142, 1941.

[6] G. W. Woolley, L. W. Law and C. C. Little, *Proc. Nat. Acad. Sci.*, 29: 22, 1942.

[7] C. P. Barnum, Z. B. Ball, J. J. Bittner and M. B. Visscher, SCIENCE, 100: 575, 1944.

[8] C. C. Little, "Biology of the Laboratory Mouse." Philadelphia: Blakiston. 1941.

[9] E. Fekete, personal communication, 1945.