TABLE IV. Acetoacetate Produced and Pyruvate Utilized by Liver Slices from Diabetic Rats.†

No. of rats	Glucose-cyclo-acetoacetate (hydrolysed) .016 M	Pyruvate .008 M	μl of oxygen uptake	Acetoacetate* produced	Pyruvate disappeared in γ
			——per 100 mg wet wt of tissue in 2 hr——		
3			128 ± 10	24 ± 3	260 ± 10
3	Added		162 ± 10	15 ± 4	260 ± 11
3		Added	174 ± 11	60 ± 7	1970 ± 30
3	Added	"	139 ± 7	20 ± 10	2155 ± 50

* CO_2 in μl produced after decarboxylation of acetoacetate with aniline citrate.
† Alloxan diabetic rats (2 wk)—urine sugar 3%. Air was used as gas phase in vessels.

liver. (2) These substances cause more utilization of added pyruvate, both by liver slices and diaphragm. (3) They have no effect on interconversion of acetoacetate and B-hydroxybutyrate.

1. Nath, M. C., Brahmarchari, H. D., *Nature,* 1944, v154, 467.
2. ———, *ibid.,* 1948, v161, 18.
3. Nath, M. C., Chakrabarti, C. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 326.
4. ———, *Ind. J. Physiol. and Allied Sci.,* 1951, v5, 43.
5. Nath, M. C., Sahu, V. K., Chitale, R. P., *Biochem. J.,* 1953, v53, 684.
6. Nath, M. C., Hatwalne, V. G., Gadgil, J. S., *ibid.,* 1953, v53, 481.
7. Nayudu, S. G., Mookerjea, S., Nath, M. C., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 179.
8. Nath, M. C., Sahu, V. K., *Metabolism,* 1956, v5, 3.
9. Nath, M. C., Bhattathiry, E. P. M., *ibid.,* 1956, v5, 11.
10. Nath, M. C., Behki, R. M., *ibid.,* 1956, v6, 127.
11. Nath, M. C., Sahu, V. K., Behki, R. M., *ibid.,* 1956, v5, 18.
12. Nath, M. C., Behki, R. M., *Arch. Biochem. and Biophysics,* in press.
13. Chaikoff, I. L., Soskin, S., *Am. J. Physiol.,* 1940, v130, 144.
14. Raper, H. S., Smith, E. C., *J. Physiol.,* 1926, v62, 17.
15. Jowett, M., Quastel, J. H., *Biochem. J.,* 1935, v29, 2159.
16. Edson, N. L., *ibid.,* 1935, v29, 2082.
17. Evans, E. A., *ibid.,* 1940, v34, 829.
18. Lehnninger, A. L., *Lipid Metabolism, Biochemical Symposia,* 1952, v9, 66.
19. Crandall, D. I. Gurin, S., *J. Biol. Chem.,* 1949, v181, 829.
20. Stadie, W. C., Zapp, J. H., Luckens, F. D., *Trans. Assn. Ann. Phys.,* 1940, v55, 247.
21. Umbreit, W. W., Burris, R. H., Stauffer, J. F., *Manometric Technique and Selected Methods for Study of Tissue Metabolism.* Minneapolis. Burgess, 1945.
22. Friedman, T. E., Haugen, G. E., *J. Biol. Chem.,* 1943, v147, 415.
23. West, E. S., *ibid.,* 1927, v74, 561.
24. Nath, M. C., Chitale, R. P., Belavady, B., *Nature,* 1952, v170, 545.
25. Villee, C. A., Hastings, A. B., *J. Biol. Chem.,* 1949, v181, 131.

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Harmonic Analysis of the Ballistocardiogram. (23854)

CARL R. HONIG AND WOLF W. VON WITTERN (Introduced by Wallace O. Fenn)
Departments of Physiology and Medicine, University of Rochester School of Medicine and Dentistry, Rochester, N. Y., and The Aero-Medical Laboratory, Wright-Patterson Air Force Base, O.

The two mass analysis upon which modern ballistocardiography is based can only be verified for low frequencies(1,2). Above approximately 15 CPS, platform, trunk and limbs no longer move in unison, and the influence of the coupling between heart and skeleton is markedly increased(3,4). On the other hand, frequencies up to 35 CPS may be required to distinguish sequential physiologic events(5, 6). It is therefore important to determine the ballistocardiographic frequency spectrum undistorted by body resonance. In this report

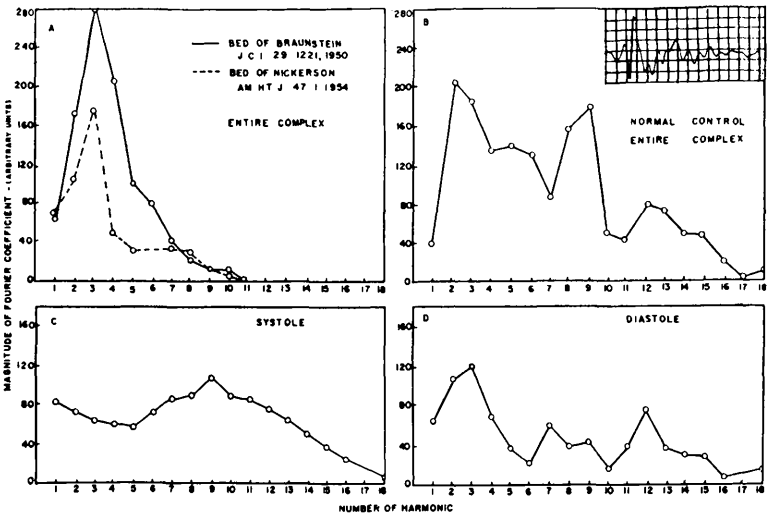

FIG. 1. Fourier analysis of ballistocardiograms recorded with high and low frequency beds (A), and an ultra-low-frequency bed (B, C, and D).

Fourier analyses of high, low, and ultra-low frequency ballistocardiograms are compared, and contrasted with Fourier analyses of aortic flow pulses(7,8). Since the ultra-low-frequency system employed has a theoretically flat frequency response to 35 CPS, the data indicate the relative magnitude of reaction forces between 1 and 20 CPS(2).

Methods. The ballistocardiograph consists of a rigid 16 lb platform suspended with appropriate damping to a natural frequency of 0.5 CPS. Platform acceleration is measured directly, and the band 0.5-25 CPS selected with a Krohn-Hite filter model 330A; filter cut-off slope is proportional to 1 f^2(2,5). A periodic time function can be represented as the sum of sinusoidal waves of various amplitudes, phase relations and frequencies, in the case of the ballistocardiogram the heart rate and its integer multiples. Such a breakdown is called a Fourier analysis. Twelve by 20 inch photographic enlargements of representative beats recorded from each of 5 normal subjects were examined with a planimetric harmonic analyser, and Fourier coefficients for harmonics 1-25 were determined. Systolic and diastolic deflections are originated by separate physiologic events and involve different anatomic structures(5,6). Frequency spectra were therefore obtained for systole and diastole individually as well as for the entire cycle. To this end systole, diastole and the complete cycle were separately traversed with the analyser, retaining the full cycle length as the fundamental. Diastole was assumed to begin with the ballistic deflection coinciding with the end of the electrocardiographic T wave. This approximation has been validated by subsequent experiments in which the ballistocardiogram and electrocardiogram were recorded simultaneously with central flow and pressure pulses(5,6). The spectrum of the entire complex is not the sum of its systolic and diastolic components as measured because of phase conditions.

Results. Fourier analyses of high and low frequency displacement ballistocardiograms obtained from references 7 and 8 are replotted as Fig. 1A. Both exhibit resonance at 3 CPS, the natural frequency of the unrestrained body moving on its panniculus. Even twice differentiating these curves does not bring out significant components above 10 CPS. Both Nickerson and Braunstein appreciated the fact that their records were distorted by the vibration properties of the body, and Nickerson correctly concluded that forces to at least 11 CPS were important to the acceleration record(7,8).

Fourier analyses of an ultra-low-frequency ballistocardiogram are presented in Fig. 1B, C, and D, together with the oscillographic record from which the data were derived. Note that the frequency spectrum of systolic

forces, Fig. 1C, exhibits no 3-cycle peak. Since resonance is independent of the origin of the driving forces, such a peak should appear in the systolic spectrum if the 3-cycle diastolic peak were due to resonance. Significant frequency components exist to at least 20 CPS in recordings from all 5 subjects, a finding which would be improbable if resonance were not eliminated. We therefore conclude that the ultra-low-frequency ballistocardiogram is indeed free of the vibration properties of the subcutaneous tissues, and that the band 0.5-20 CPS is the minimum, though not necessarily optimum, frequency range over which the acceleration ballistocardiogram must be recorded without phase or amplitude distortion.

Systolic and diastolic spectra are completely different and vary independently during hemodynamic change, indicating that diastolic vibrations represent new forces rather than a dying out process. For example, nitroglycerine, 0.32 mg sublingually, increases heart rate and ballistic amplitude and decreases arterial pressure and the magnitude of high frequency reaction forces. Phenylephrine hydrochloride, 5 mg intramuscularly, decreases ballistic amplitude and heart rate but increases arterial pressure and high frequency components. Parallel change in arterial pressure and the magnitude of high frequency early diastolic ballistic forces suggest that aortic valve closure contributes to the genesis of the diastolic complex. Since ballistic amplitude and Fourier spectrum may vary independently, harmonic analysis may prove clinically useful as a diagnostic criterion.

Evidence from this and other laboratories indicates that the ballistocardiogram is largely the result of acceleration of a central arterial volume(3). Through the courtesy of Dr. E. Wetterer, we obtained canine aortic velocity pulses recorded with an electromagnetic flowmeter. Fig. 2 compares the frequency spectrum of such a pulse and of its time derivative with spectra of human velocity and acceleration ballistocardiograms. This is justified because central flow pulses and ultra-low-frequency ballistocardiograms are morphologically comparable in dog and man(5).

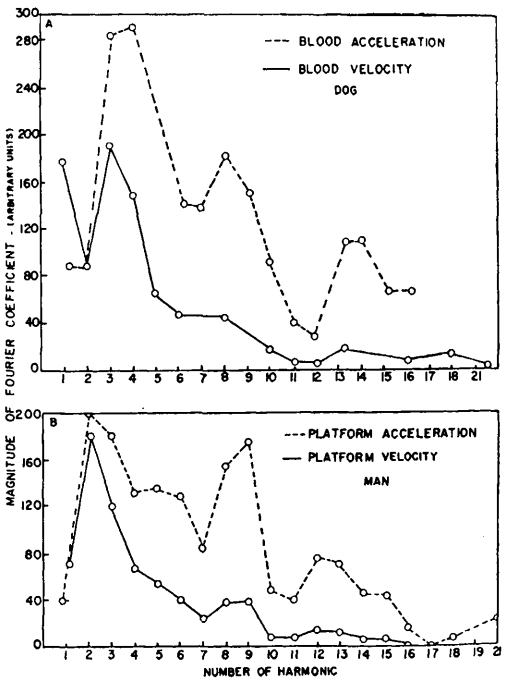

FIG. 2. Comparison between frequency spectra of canine blood flow velocity and acceleration pulses and human velocity and acceleration ballistocardiograms.

The striking similarity between the frequency spectrum of blood and platform motion supports the view that the ballistocardiogram is related to central blood flow.

Summary. Ballistocardiograms of the Starr and Nickerson type exhibit prominent resonance peaks, and contain no significant frequency components above 10 CPS. Ultra-low-frequency ballistocardiograms are distorted by body resonance to a far lesser degree, and exhibit components to at least 20 CPS. The band 0.5-20 CPS is therefore the minimum though not necessarily optimum frequency range for force ballistocardiography. The effects of respiration and vasoactive drugs suggest that harmonic analysis may prove useful as a diagnostic criterion for clinical ballistocardiography. Frequency spectra of blood velocity and acceleration pulses and of velocity and acceleration ballistocardiograms are strikingly similar, suggesting a cause and effect relationship between these wave forms.

1. von Wittern, W. W., Wright Air Development Center Tech. Rep. 1952, #52-340.

2. ———, *Am. Heart J.*, 1953, v46, 705.

3. Talbot, S. A., Harrison, W. K., Jr., *Circulation,* 1955, v12, 1022.

4. Honig, C. R., Tenney, S. M., *Am. Heart J.,* 1957, v54, 678.

5. ———, *ibid.,* 1956, v52, 167.

6. ———, *ibid.,* 1957, v53, 655.

7. Nickerson, J. L., Mathers, J. A. L., *ibid.,* 1954, v47, 1.

8. Braunstein, J. R., Oelker, C. E., Gowdy, R. C., *J. Clin. Invest.,* 1950, v29, 1219.

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Localization of Thyroid and Spinal Cord Autoantibodies by Fluorescent Antibody Technic.* (23855)

ERNST H. BEUTNER, ERNEST WITEBSKY, NOEL R. ROSE AND JOSEPH R. GERBASI
Department of Bacteriology and Immunology, University of Buffalo School of Medicine

Recently production of autoantibodies against constituent(s) of the thyroid gland, presumably thyroglobulin, has been reported from this laboratory(1-6). Highly specific autoantibodies have been observed in several experimental animal species, such as rabbits, dogs, and guinea pigs, as well as in human beings suffering from certain types of chronic thyroiditis. Concurrently, characteristic changes in thyroid gland were frequently found. Demonstration of paralysis by immunization of experimental animals with brain substance has created considerable interest(7-9). In many instances brain-specific circulating antibodies were found in serum of these animals by both complement fixation and precipitation tests. However, antibody formation against brain substance including that of antibody-producing animal has been noted in absence of observable paralysis. On the other hand, brain lesions have been observed repeatedly in absence of detectable circulating antibodies. Therefore, relationship of circulating antibodies to what is frequently referred to as *allergic encephalomyelitis* is still a moot question. The fluorescent antibody technic of Coons and Kaplan(10) seemed to offer an interesting approach to the study of the possible direct combination of circulating antibodies and tissue sections. This technic might shed some new light on specific localization of tissue-specific antibodies. Simultaneous studies were therefore undertaken of these 2 autoantibody systems with Coons technic since they seem to constitute perfect controls for each other.

Methods. Sections of quick frozen tissues were cut 6 to 8μ thick with standard microtome in a cryostat according to method of Coons and Kaplan. The air-dried sections were fixed in formalin or in formalin followed by acetone. Formalin fixation was carried out by immersion in 10% formalin in pH 7.2 buffered saline solution at room temperature. Thyroid sections could be fixed in formalin for 2 to 10 minutes. Formalin fixation followed by 10 min. acetone fixation also gave satisfactory results. However, 2-minute formalin fixation was used in most experiments. Brain and spinal cord sections, on the other hand, were fixed in formalin for only 2 minutes because they lost their capacity to stain by fluorescent antibody technic when fixed in formalin for longer than 2 minutes or when 2 minute formalin fixation was followed by acetone fixation. Rabbit antisera to homologous brain or spinal cord antigens were prepared by intracutaneous injection of 20% aqueous suspensions in equal volume of Freund adjuvant. Aqueous suspensions were prepared by homogenizing fresh brain or spinal cord in Servall omnimixer for 30 seconds at maximum speed and centrifuging 20 seconds. As source of thyroid autoantibodies anti-rabbit thyroid rabbit sera were used from the same series as in previous studies(3,4,5). Complement fixation tests for thyroid autoantibodies and spinal cord autoantibodies as well as tanned cell hemagglutination tests with experimental thyroiditis sera were carried out as described previously(1,11). Evaluations of histologic

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