étaient reprises pendant 2 semaines à la même cadence; 8 à 10 jours après la dernière injection, les animaux étaient saignés.

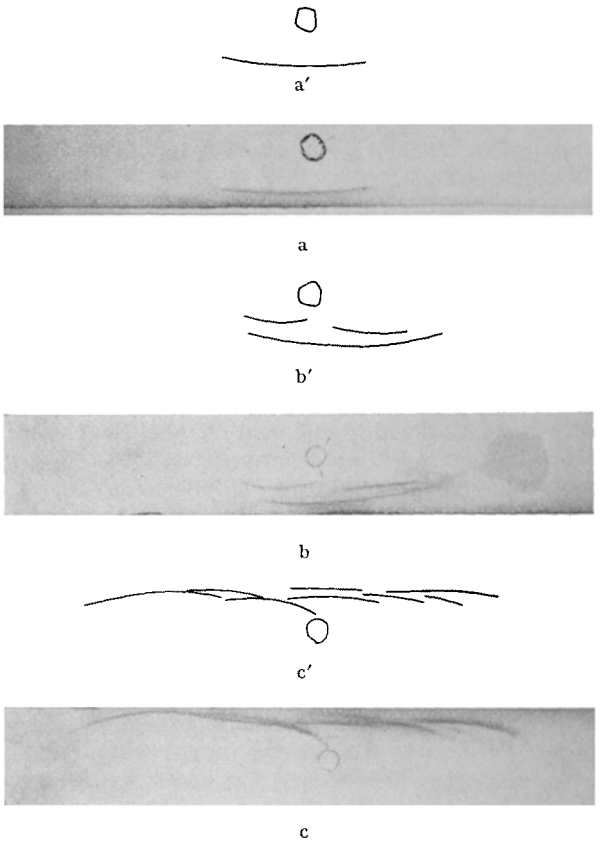


Fig. 2. Immunoélectrophorèses de gonadotropines choriales humaines contre un immunsérum antigonadotropines
(*a:* hormone titrant 12000 UI/mg);
(*b:* hormone titrant 6000 UI/mg);
(*c:* hormone titrant 1500 UI/mg).

Les immunoélectrophorèses ont été effectuées selon la méthode de GRABAR et WILLIAMS[3] modifiée en microtechnique par SCHEIDEGGER[4]; toutefois, la longueur des fentes dans lesquelles on dépose l'immunsérum était augmentée, afin d'obtenir une meilleure séparation des lignes de précipitations: au moyen d'un appareil décrit précédemment[5], des fentes de 6 cm étaient ainsi préparées.

Toutes les immunoélectrophorèses étaient réalisées en tampon véronal à pH 8,2.

Résultats. Cette technique permet de déterminer le nombre des constituants antigéniques présents dans l'échantillon examiné, et de les caractériser d'après leur mobilité électrophorétique et leur mobilité de diffusion (distance du sommet de la ligne de précipitation à la fente contenant l'immunsérum).

Une première série d'immunoélectrophorèses a été effectuée (Fig. 1) contre un immunsérum de cheval anti-sérum humain (sérum commercialisé par l'Institut Pasteur).

La préparation titrant 1500 UI par mg donne deux lignes de précipitation, au niveau de l'albumine et des globulines α_1 ainsi qu'une légère traînée en β (Fig. 1*b*) alors que la gonadotropine pure ne donne aucune ligne (Fig. 1*a*), ce qui était prévisible puisqu'elle ne se trouve pas dans le sérum normal (la Figure 1*c* est un cliché d'une immunoélectrophorèse du sérum humain normal, effectué à titre de comparaison).

Contre l'immunsérum de lapin antigonadotropine que nous avons obtenu, la préparation titrant 1500 UI par mg, ayant servi à le préparer, montre 7 à 8 lignes, de mobilités variables (Fig. 2*c*). Une préparation déjà très active (6000 UI par mg) ne montre plus que 4 lignes (Fig. 2*b*) détectant ainsi les impuretés de ce produit; enfin une seule ligne apparaît pour l'hormone pure (Fig. 2*a*): cette ligne se situe entre les lignes des globulines α_2 et β_1 (la mobilité électrophorétique de la gonadotropine pure est à pH 8,6, de $-2{,}5 \times 10^{-5}$)[6], cependant sa courbure est moins accentuée et sa diffusion est plus rapide.

Remarquons que cette ligne caractéristique se retrouve dans les hormones impures.

De cette série d'expériences, les conclusions suivantes peuvent être tirées: d'une part, la pureté de la préparation hormonale titrant 12000 UI par mg est confirmée par une méthode d'une grande sensibilité; d'autre part, la gonatropine choriale humaine présente des propriétés antigéniques caractéristiques qui peuvent être utilisées pour suivre les progrès d'une purification, et vérifier facilement le degré de pureté des hormones obtenues.

R. GOT, GEORGETTE LEVY et R. BOURRILLON

Laboratoire de Chimie Biologique, Faculté de Médecine, Paris, le 13 juillet 1959.

Summary

The purity of human chorionic gonadotropin showing an activity of 12,000/mg, is confirmed by immunoelectrophoretic analysis. Characteristic antigenic properties of this hormone are revealed by this method.

Ozone Production by a Corona-type Loudspeaker and its Toxic Effects in Mice[1]

In previous communications[2], a high frequency (1-50 kc), high power (1·3 kw) corona-type loudspeaker (Ionophone[3]) was described as useful for studies of biological effects of noise in animals. The present report points out the fact that such speakers are also very efficient ozonizers and should not be used without adequate ventilation.

In principle, the Ionophone is a thermal sound source. The apparatus used in the present work consists of an audio signal generator, a radiofrequency oscillator, and a Vycor glass horn with a central platinum electrode and an outer electrode. By means of a step-up transformer, a radio-frequency corona is produced between the center and outer electrodes. The rf power source can be modulated by the audio signal, and the thermal energy of the corona is then modulated in the signal frequency range, 2–40 kc. Sound is produced as a result of adiabatic pressure changes which the corona produces in the air. Both

[3] P. GRABAR et C. A. WILLIAMS, Biochem. Biophys. Acta *10*, 193 (1953).

[4] J. J. SCHEIDEGGER, Int. Arch. Allergy *7*, 103 (1955).

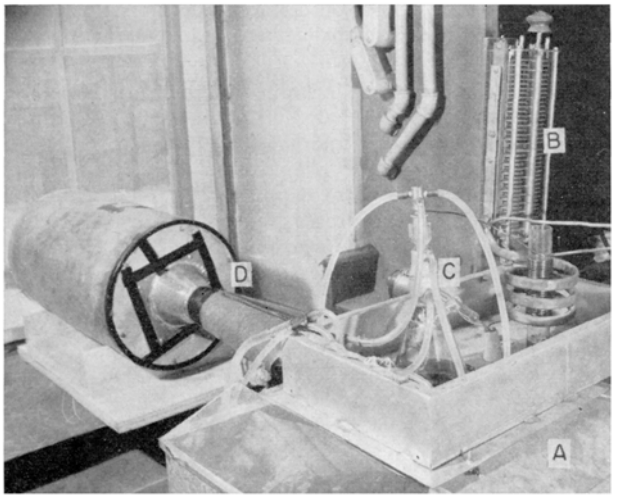
[5] G. LEVY et J. POLONOVSKI, Bull. Soc. Chim. biol. *40*, 1293 (1958).

[1] Paper No. 2397 in the Journal Series of the Agricultural Experiment Station.

[2] E. ACKERMAN, A. ANTHONY, and F. ODA, Exper. *14*, 384 (1958). F. ODA, Corona Type Loudspeaker (WADC Technical Report 58 368, Wrinht Air Development Center, Dayton, Ohio 1958).

[3] S. KLEIN, Acoustica *4*, 77 (1954).

water and forced air cooling systems were employed to prevent overheating of the electrodes and horn.



Close up of Corona Type Loudspeaker Mounted on Roof of Reverberant Exposure Chamber

(*A*) Reverberant chamber; (*B*) Speaker assembly with hoses for water and air cooling; (*C*) Step-up coil circuit and matching condensor; (*D*) Ventilating system

The Ionophone was mounted on a small reverberant chamber (16 × 20 × 12 in.) as shown in the Figure. The exhaust fan shown on the left was added after analyses of ozone levels were completed. The noise level during exposure was 132 db (re 0·0002 dynes/cm^2) in the frequency band, 2–40 kc. Single, 15 min exposures of mice to Ionophone noise were employed.

The first tests consisted of subjecting mice singly or in groups of 15 to Ionophone noise for 10 to 15 min. In all instances the mice died during the course of exposure, or within a few hours. During these studies, considerable ozone odor could be detected in the reverberant chamber as well as in the laboratory which housed the apparatus. Following these tests, there was also a noticeable increase in complaints of headaches and throat irritation among laboratory personnel. In order to verify that the ozone, and not intense noise, was the toxic factor involved, the Ionophone was permitted to run for 10 min and then turned off. Fifteen mice were brought into the laboratory and placed into the ozonized atmosphere of the exposure chamber without subjecting the mice to any noise stimulation. All of the mice again died within a short time after introduction into the chamber. This was repeated on several occasions and in all cases mice died showing the same symptomatology that was evidenced in the earlier tests with noise stimulation.

Three stages of ozone intoxication were discernible in these mice: (a) a stage of hyperexcitation, characterized by restlessness, leaping of mice within the chamber and some fighting; (b) a stage of depression, which occurred within 15 min after exposure, wherein the mice became inactive and showed signs of dyspnea, and (c) a stage where mice exhibited extreme dyspnea, uncoordinate movements (usually followed by partial paralysis of the hind legs), coma, and death. Mice which survived for several hours showed severe mucous discharge from the nose and mouth. At autopsy, all mice showed very marked hyperemia and hemorrhage of the respiratory tract associated with severe pulmonary emphysema.

Ozone concentrations were measured in the reverberant chamber and in the laboratory using the method recommended by BOELTER *et al.*[4]; as a check, measures were also made following the procedure outlined by BIRDSALL *et al.*[5]. Both methods involve the oxidation of KI to I_2 by the ozone and the subsequent titration of free I_2 with $Na_2S_2O_3$. All of the values obtained within the exposure chamber (10 min running time of the Ionophone) were several hundred-fold the lethal concentrations reported for animals or humans[6]. The values ranged from 500 to 4100 ppm (parts per million) of ozone in the exposure chamber. In the laboratory, outside the chamber, the ozone level was about 3–8 ppm after the Ionophone was operated for 10–15 min.

By simply using forced exhaust ventilation (7 in. rotary fan, 1700 rpm), piped through a window to the outside, the ozone level within the chamber and laboratory was reduced below the limit detectable by odor, or measureable by the iodometric techniques employed ($< 0{\cdot}1$ ppm). Three dozen mice which were subjected to Ionophone noise stimulation (with ventilation) for 17 h a week over a period of 2–1/2 weeks showed no symptoms of ozone intoxication or evidence of respiratory pathology.

Some authors list the minimum allowable concentration of ozone for humans as near the level at which ozone can be detected by odor (0·05–0·1 ppm)[6]. The toxic limit (throat irritation, headaches) is thought to be about 1 ppm for several hours exposure[7]. The lethal limit is not specified exactly for man, but for animals is thought to be about 15 ppm for several hours exposure[8]. In an excellent review of the ozone literature, THORP recommends that exposures to ozone concentrations in excess of 1 ppm should be considered as potentially hazardous to health[9].

In recent years, high fidelity, low power, corona-type speakers have been advertized as excellent, very-high frequency tweeters. In view of the fact that such tweeters may also be excellent ozonizers, one should caution against using these in poorly ventilated places, especially if ozone odor is detectable.

A. ANTHONY, S. BABCOCK, and F. ODA

Biophysics Laboratories, Department of Zoology and Entomology and Department of Physics, The Pennsylvania State University, University Park (Pennsylvania), July 29, 1959.

Résumé

L'ionophone est un émetteur sonique et ultra-sonique qui utilise la particularité qu'ont les gaz d'être ionisés sous l'effet d'une tension électrique très élevée et de haute fréquence. Ce générateur est aussi un ozoniseur efficient et il est nécessaire de ne l'employer qu'avec une ventilation appropriée.

[4] E. D. BOELTER, G. L. PUTNAM, and E. I. LASH, Analyt. Chem. *22*, 1533 (1950).

[5] C. M. BIRDSALL, A. C. JENKINS and E. SPADINGER, Analyt. Chem. *24*, 662 (1952).

[6] S. WILSKA, Acta chem. scand. *5*, 1359 (1951).

[7] C. E. THORP, Chem. Eng. News *19*, 686 (1941). – C. E. THORP, Ind. Med. Surg. *19*, 45 (1950).

[8] A. C. GIESE and E. CHRISTENSEN, Physiol. Zool. *27*, 101 (1954).

[9] C. E. THORP, *Bibliography of Ozone Technology*, vol. 2. *Physical and Pharmacological Properties* (Armour Research Found., Illinois Inst. Tech., Chicago, Illinois 1955).