REVISTA ESPAÑOLA DE FISIOLOGIA
R. esp. Fisiol., 15, n.º 1, págs. 13-22, 1959

Departamento de Bioquímica de Madrid
Instituto Español de Fisiología y Bioquímica
(Madrid)

Polarography of blood serums and of serum mucoproteins

by
J. A. Cabezas, J. Lucas-Gallego and A. Santos-Ruiz

(Recibido para publicar el 29 de noviembre de 1958)

In the course of the last years, intensive work has been made in several research centers on the interesting subject of blood serum polarography and its application to the clinical diagnosis.

The Department of Biochemistry of the «Instituto Español de Fisiología y Bioquímica» has been working on this problem since 1945. In view of the fact that many publications have been made recently on this subject, we believe it convenient to give some idea of our work and to quote some of the experiments we have carried out here.

In the sulphosalicylic filtrates of blood serum from cancer patients, and from some other pathological processes, the polarographic wave is higher than in normal serum. [BRDICKA (3), 1939; CROSSLEY, 1941; WINZLER (14), 1944; SANTOS-RUIZ and LUCAS-GALLEGO (12), 1945.]

The technique used by SANTOS-RUIZ and LUCAS-GALLEGO is Brdicka's technique, i.e. alkaline denaturation with KOH N/10, deproteinization with a solution of sulphosalicylic acid at 20 % and polarography in cobalt-hexamin medium. This technique proved that:

a) In serums taken from cancer, tuberculosis, typhus and paratyphoid fevers, the double wave was higher than in serums taken from normal subjects; *b*) the height of the double

wave decreases when the tumour is taken out or if the patient is better or cured. It decreases until reaching the height of the polarogram of normal serum ; *c)* in case of relapse or metastasis, there is an increase in the height of the double polarographic wave.

LUCAS GALLEGO, SANTOS-RUIZ and JACOB ERNST (7) (1951) have carried out experiments on the mecanism of the formation of the polarographic wave in blood serum. They have polarographied : 1) fresh normal and pathological serums ; and 2) the same serums incubated with or without addition of chemicals. The incubation took place at 0° C and at 37° C during a variable period of time from 1 to 72 hours. Glutathione and cystine were added to the serum. These two chemicals did not modify the height nor the form of the double wave. In the normal serum treated with glutathione or cystine, and incubated at 37° C during 20 hours, it was observed that the height of the double wave was bigger than for serums which had not been incubated.

When glutathione or cystine were added to the pathological serum (cancer and tuberculosis) and when they were incubated at 37° C during 20 hours, the height of the double wave was the same as when the incubation did not take place.

In experiments carried out with incubation for a period of time of 48 hours, the double wave increased in the case of normal serum, but it dit not vary for serum taken from patients suffering from cancer.

The increase of the double wave, in experiments carried out on normal serums incubated at 0° C, during 20 hours, was less than when incubated at 37° C.

When blood serum was treated with cystine and incubated at 37° C for 20 hours, the increase of the peak of the double wave was, in the case of normal serums, bigger than when gluthatione was used. The same result was obtained when incubation was carried out at 0° C.

From these results, it seems to be certain that the addition of either glutathione or cystine to the normal blood serum causes an increasing amount of polarographycally active substances in the filtrate ; this is possibly due to an enzymatic activation. This activation does not take place in cancer serum.

Besides, the heights of the wave are : Cancerous > Tuberculous > Normal. (Fig. 1.)

JACOB ERNST, LUCAS GALLEGO and SANTOS-RUIZ (5) (1950), found that, when adding tyrode liquid to the solution of cobalt-

hexamine, the form of the polarographic curve was not modified. However the cobalt wave was shorter. Identical results were obtained when adding glucose. Glutathione in its two forms (oxidized and reduced) and cystine affected the maximum of the cobalt in the polarograms. Its height decreased and at the same time became more round, without affecting the prenatrium.

RODRÍGUEZ ORTEA, SANTOS-RUIZ and LUCAS-GALLEGO (11), (1951), carried out new polarographic analysis on serums from patients suffering from cancer, syphilis and tuberculosis and also on normal serums. The serums were incubated with glutathione at 37° C for 24 hours. In the course of these experiments, the following was observed :

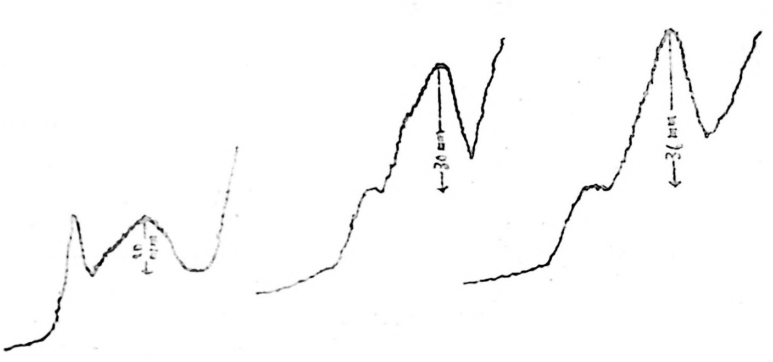
a) The polarographic curve of the normal blood serum is higher if the serum is incubated than if is not ; *b*) In most cases, the peak of the double wave of the cancerous blood serums does not change when incubation takes place ; *c*) In blood serums of syphilitic patients, the low double wave decreases when incubation takes place ; *d*) The high double wave of the tuberculous blood serum is slightly inhibited when incubation takes place.

BALLE-HELAERS and DE PADUWA (1) (1954) in experiments similar to those described before, divide the serum into three fractions which they denaturate with KOH, during 0, 30 and 60 minutes. They have thus been able to show that in normal serum the height of the double wave varies very little in the experiments at 30 min. and at 60 min., in comparison with same at 0 min. ; in tuberculosis serums, the height of the double wave increases at 30 min. and at 60 min. also, though less remarkably ; in cancer serums the height at 30 min. increases when compared with the one at 0 min., and after 30 minutes the increase of the double wave is small ; however it is considerably higher at 60 min (*). The two abovenamed authors pretend that their technique may be utilized for the early diagnosis of cancer, and suggest the hypothesis, already proposed by SANTOS-RUIZ and his co-workers, that sulphidrilic substances are liberated through enzymatic actions in the course of the process.

BALLE-HELAERS (2) (1956), points out the slight modification which occurs in the polarographic wave of the solution

(*) Besides, the heights of the wave are (at 60 m.) : Cancerous> Tuberculous> Normal.

of cobalt-hexamine when a solution of gelatine is added, as well as the disappearance of the cobalt maximum. With tylose-cistamine in solution of cobalt-hexamine, BALLE-HELAERS gets a double wave similar to the one given by proteins. The higher the concentration of those sulphidrilic substances, the higher the double wave. The second wave of the polarographic curves of blood serums would be a function of the concentration of



a) b) c)

Figura 1

a) normal
b) tuberculous
c) cancerous

tylose-like substances which would lose activity by denaturation. It presents abnormal polarographic waves which can appear in curves from patients suffering from other serious diseases. These abnormalities are less striking in non-cancerous patients if the patient's general condition gets better; it may therefore be concluded that polarography may be applied to the early diagnosis of the disease.

As we can see from the above-mentioned, BALLE-HELAERS' works show, in some aspects, a coincidence with our priorly-obtained results. And it appears clearly that the alkaline denaturation method on blood serum and its polarography in medium of cobalt-hexamine is useful for diagnosis of cancer, for early diagnosis of malignancy as well as for differential diagnosis of other diseases, although it lacks of the specificity it should have to become a really valuable method.

The utilization of glutathione prior to incubation may help in a greater diagnostic differenciation.

Referring to the biochemical mechanism of the formation of the polarographic wave, here is a summary of some of the experiments we carried out here :

CABEZAS FERNÁNDEZ DEL CAMPO (3) (1956), separated the mucoproteic fraction from normal serum (ANDERSON-MACLAGAN'S method) and polarographied this fraction. He obtained a wave whose form and characteristics were almost similar to those of Brdicka's filtrates. This wave is produced by saturated dissolutions of mucoproteins of *p*H 9.5, in quantities inferior to those generally utilized of Brdicka's filtrate. (See Fig. 2.)

While studying the polarographic influence of glutathione added to the mucoproteins and to Brdicka's filtrate, he noticed that the aforementioned tripeptide causes an increase in the height of the wave of mucoproteins, as well as in the wave of the filtrate. This increase varies with the temperature and with the time of incubation. It is bigger at 37° C than at 0° C, and it is during the first six hours that the most important variations take place.

The treatment with KOH 0.1 N (at 20° during 45 min.) has practically no influence on the mucoproteins, as far as an increase or decrease of the height or the modification of the typical wave is concerned. The supernatant liquids of the precipitation of the serum mucoproteins do not produce either the characteristic wave.

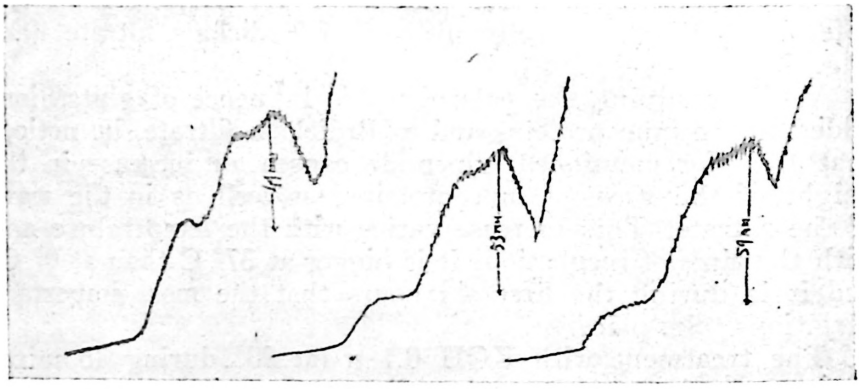
CABEZAS FERNÁNDEZ DEL CAMPO (3) (1956) compared the contents of nitrogen, ashes, etc., of the mucoproteins and of Brdicka's filtrate. He found considerable differences between both, in spite of their similar polarographic wave, which can be explained by the catalytic nature of the polarographic wave. Nevertheless he found tha the mucoproteidic substances always pass, at least partially, in Brdicka's filtrate. It finally results that SH-groups (belonging to the mucoproteidic substances and to Brdicka's filtrate) linked to more complex substances, form the active polarographic compounds which are responsible for the formation of the typical wave.

The experiments made by ROBERT, SERPICELLI, and JAYLE (9) (1956) confirm our previous studies on the value of the polarographic test in the diagnosis of cancer, even if such value is limited because of its lack of specificity.

On the other hand, JAYLE, SERPICELLI and ROBERT (9) (1956) have studied the correlation between the haptoglobin

titer (a_2-seromucoid) and the three parameters of the polarographic test of the sulphosalicylic filtrate, which characterizes the a_1-seromucoid [height of the wave = h ; difference in μA between the second wave obtained after treatment with KOH and the wave obtained without KOH, (alkaline effect) ; and, difference between the two waves].

They have come to the conclusion that there is a good correlation between the height of the wave, h, and the hapto-



a) *b)* *c)*

Figura 2

a) Brdicka's filtrate
b) Impurified seromucoid
c) Purified seromucoid

globin titer of normal serums and non-cancerous serums, but not in cancerous serums. These studies confirm the possibility of dissociation of the values of a_1 and a_2 seromucoids in certain diseases.

ROBERT, MARNAY, SERPICELLI and JAYLE (10) (1957) show that between the perchlorosoluble mucoid titer (determined by the turbidometric method of POPER, DE LA HUERGA and collaborators) and the height of the polarographic wave of the sulphosalicylic filtrate, it does not exist a very narrow correlation, i. e. the height of the wave varies not only with the perchlorosoluble mucoid titer of the serum, but also with other seric substances, such as fatty acids and glycerids ; it also varies with differences in the structure and composition of the mucoids, due to certain diseases.

CABEZAS, VELAMAZÁN, BELLOT and LUCAS (4) (1958) have

recently studies the polarographic curves of filtrates of serums proceeding from normal dogs operated of gastric or intestinal resection or extirpation of gall-bladder (according to the series) ; they have observed that the height of the typical wave is smaller when the serum is taken at the very moment of the anaesthesia — made with pentotal and ether-oxygen — than when it is taken : a) a few days before, or b) even some minutes before the operation, or c) when it is taken after the animal has been operated and has recovered. In other words, the heights are practically equal to one another in the case of serums taken before and after the anaesthetic phase and bigger than the values obtained during the said phase.

On the other hand, the contents in blood glutathione show modifications corresponding to the above mentioned modifications in the height of the wave.

At the present moment we are continuing to study the decisive influence of the mucoproteins on the height of the polarographic wave. It is our intention to extend our analysis to mucoproteins proceeding from pathological serums and urines.

Lately, SERPICELLI and ROBERT (13) (1958) have worked on kinetics of alkaline denaturalization of normal and pathological serums. They use, amongst other determinations, the measure of the height of the polarographic wave in cobaltic buffer. The abovementioned authors also confirm the fact that this wave increases in some pathological serums during the denaturalization process, although the contents in mucoproteins of the sulphosalicylic filtrate decreases for loss of solubility.

LUCAS GALLEGO (8) (1958) admits that Medicine represents a vast field of investigation for Polarography ; for this reason, and according to him, polarography should be added to the methods normally used in laboratories, not only as an auxiliary in diagnosis, but also in order to help to follow the evolution of the different processes.

Resumen

Se recogen los resultados principales de una serie de trabajos, iniciados ya en 1945, referentes a la polarografía de sueros sanguíneos y sus aplicaciones clínicas, efectuados por equipos de nuestro laboratorio y por otros investigadores que, independientemente de nosotros, han trabajado sobre el mismo tema y cuyas conclusiones se hallan en relación con las nuestras.

De estos estudios se deduce :

1.º El tiempo y temperatura de incubación y la adición de sustan-

cias portadoras de grupos —SH favorecen, entre ciertos límites, el aumento de la altura de la curva polarográfica producida por el filtrado sulfosalicílico.

2.º Separados los mucoproteidos séricos o efectuada una desnaturalización alcalina en el suero, dichos mucoproteidos producen la onda típica, aun hallándose en el filtrado sulfosalicílico a concentración inferior a la que se encuentran en el suero.

3.º La diferencia de alturas entre sueros patológicos y normales, aumentada por ciertos tratamientos tales como desnaturalización alcalina progresiva, incubación con glutatión a 37º, etc., hace que este método, aun careciendo de especificidad rigorosa, sea útil en el diagnóstico del cáncer.

Summary

We have summarized the main results of a series of studies, started in 1945, referring to the polarography of blood serums and its clinical applications. These studies have been realized by teams of our Laboratory and other investigators who, independantly from us, have worked on the same problem, and whose conclusions are in connection with ours.

From these research works, the following conclusions can be reached :

1.º Time and temperature of incubation, as well as addition of substances with —SH groups, helps, (within certain limits), the increase of the polarographic curve height produced by the sulphosalicylic filtrate.

2.º When the seromucoproteins are separated, or if a alkaline denaturalization of the serum takes place, the mucoproteins involved produce the typical wave, although their concentration in the sulphosalicylic filtrate is inferior than in the serum.

3.º The difference of heights between the pathological and normal serum (increased by some treatments, such as progressive alkaline denaturation, incubation with glutathione at 37º etc.) makes this method useful in the diagnosis of cancer, in spite of its lack of strict specificity.

Bibliography

(1) Balle-Helaers, E., De Paduwa, G. : *Brux. Med.*, **34**, 2.571, 1954.

(2) Balle-Helaers, E. : *Brux. Med.*, **36**, no. 7 & 8, 1956.

(3) Cabezas Fernández del Campo, J., A. : *Tesis Doctoral, Fac. of Pharmacy*, Madrid, 1956.

(4) Cabezas Fernández del Campo, J. A., Velamazán, V., Bellot, E., Lucas Gallego, J. : *Report presented at the IVth Meeting of Physiological Sciences*, Granada, May 1958.

(5) Jacob Ernst, N., Lucas Gallego, J., Santos-Ruiz, A. : *R. esp. Fisiol.*, **6**, 207, 1950.

(6) Jayle, M. F., Serpicelli, J., Robert, L. : *Clin. Chim. Acta*, **1**, 432, 1950.

(7) Lucas Gallego, J., Santos-Ruiz, A., Jacob Ernst, N. : *R. esp. Fisiol*, **7**, 7, 1951.

(8) LUCAS GALLEGO, J. : *Ind Gallego-Portuguese Medical Meeting*, Vigo, Aug. 1958.

(9) ROBERT, L., SERPICELLI, J., JAYLE, M. F. : *Rev. Franc. d'étud. clin. biol.*, **1**, 976, 1956.

(10) ROBERT, L., MARNAY, A., SERPICELLI, J., JAYLE, M. F. : *Ann. Biol. Clin.*, **1**, 83, 1957.

(11) RODRÍGUEZ ORTEA, A., SANTOS RUIZ, A., LUCAS GALLEGO, J. : *R., esp. Fisiol.*, **7**, 17, 1951.

(12) SANTOS-RUIZ, A., LUCAS GALLEGO, J. : *R. esp. Fisiol.*, **1**, 203, 1945.

(13) SERPICELLI, J., ROBERT, L. : *Abstracts of Communications, IVth International Congress of Biochemistry*, Wien, Sept. 1958.

(14) WINZLER, R. J. : *J. Nat. Cancer Inst.*, IV, 417, 1944.