Canad. Med. Ass. J.
Jan. 16, 1965, vol. 92

SHORT COMMUNICATION

Incidence of Rubella Antibodies in Female Subjects

KATHLEEN F. GIVAN, M.D., Dip.Bact., K. R. ROZEE, Ph.D., Dip.Bact.
and A. J. RHODES, M.D., F.R.C.P.(Edin.), *Toronto*

ABSTRACT

Sera from females aged 1 to 40 years were assayed for rubella virus antibodies. Results showed that by age 14 years, 60% had antibodies and that by 19 years, 70% were positive. This figure rose to 80% by 24 years of age and remained unchanged in older age groups.

A comparison of the incidence of high and low levels of antibodies in each age group revealed that antibody levels fell between ages 20 and 40 years. Only 20% of individuals in the latter group had a high antibody level compared to 80% in the former.

These results are discussed as they relate to the problems of reinfection and possible vaccination procedures.

SOMMAIRE

On a recherché les anticorps du virus de la rubéole dans le sérum de personnes du sexe féminin âgées de 1 à 40 ans. Il ressort de ces essais biologiques que 60% de ces personnes avaient des anticorps à l'âge de 14 ans, et que ce pourcentage est monté à 70% à l'âge de 19 ans, et à 80% à l'âge de 24 ans, pour demeurer ensuite stationnaire aux âges plus avancés.

Si on compare la concentration des anticorps dans chacun des groupes, on constate qu'elle tombe entre les âges de 20 et de 40 ans. Des sujets de ce dernier groupe, 20% seulement avaient une concentration élevée d'anticorps, alors qu'elle existait chez 80% des sujets du premier groupe.

L'auteur discute les résultats à la lumière du risque de réinfection et de la possibilité d'une vaccination.

SINCE the isolation of the virus of German measles in 1962[1-3] it has become possible to assay serum for specific antibodies. This test can be put to good use, since the present knowledge of the true incidence of infection with rubella virus is scanty. It is well known, for example, that a clinical history of "German measles", without virological confirmation, cannot be relied upon, for enteroviruses and other viruses cause "rubelliform" rashes. It also seems likely that subclinical infection is at least as common as overt disease.[4] This paper describes the results of a serological survey of "normal" females in the area of Southern Ontario to determine the incidence of rubella antibodies. This information constitutes an important background for the national use of a rubella vaccine in an effort to prevent the far-reaching effects of rubella virus on the fetus. It is now generally realized that rubella virus not only causes congenital malformations of the eye, ear, heart and brain, as first described by Gregg in Australia a quarter of a century ago, but also causes abortion, stillbirth, and an increase in deaths in the first year of life.[5]

From the Department of Microbiology, School of Hygiene, University of Toronto.

This work was supported by a grant to the University of Toronto from Mr. E. Carey Fox for research in German measles. Dr. K. F. Givan was the holder of the Fitzgerald Memorial Fellowship of the Connaught Medical Research Laboratories, University of Toronto.

Collection of Serum Specimens

The sera used in this survey were obtained through the courtesy of Dr. L. E. Elkerton and Mr. H. F. Smith from the Central Laboratories, Ontario Department of Health, Toronto, and were submitted by private physicians from Metropolitan Toronto for routine diagnostic tests. Sera were collected from females only and were grouped according to age, from 3 months to 40 years. The sera were not inactivated by heat before use in tests for rubella neutralizing antibody.

Virus and Cells

The virus used in the experiments was the rubella strain known as R23, passed 12-15 times in grivet kidney cells. This strain was originally isolated in this laboratory.[3] This virus measured between 50 and 100 mμ. by millipore filtration, was inactivated by ether, and had a half-life at 50° C. of 72 minutes, the thermoinactivation being enhanced by the presence of 1.0 M $MgCl_2$. This strain grew well in cells from rabbit and grivet kidney and in the LLC-MK_2 line of monkey kidney cells, as shown by the initiation of interference. It was also capable of causing direct cytopathic changes in RK13 cells.[6]

The Gregory strain of ECHO 11 virus, originally obtained from Dr. Albert Sabin, Cincinnati, was

used as a challenge virus throughout the experiments to be described.

Grivet monkey kidney cells were obtained in suspension from Connaught Medical Research Laboratories (CMRL) and were grown as monolayer cultures, as reported previously.[3]

TITRATION OF INTERFERENCE-INHIBITING (NEUTRALIZING) ANTIBODY

Sera were diluted to 1:6 and 1:24 and added in equal volumes to rubella virus suspensions diluted to contain 100 50% interfering-doses (InD_{50}) per 0.1 ml. The diluent for both virus and serum was Earle's balanced salt solution containing 0.5% lactalbumin hydrolysate and 0.1% yeast extract.

The serum-virus mixtures were incubated at room temperature for one hour. Then 0.2 ml. of the mixture was inoculated into each of four grivet kidney monolayer cell cultures in 16 x 125 mm. tubes, from which the culture medium had been previously drained. Adsorption was allowed to proceed for 30 minutes at room temperature and then 0.8 ml. of maintenance medium (CMRL) medium 597 + 2% sheep serum was added.

Controls consisted of cultures inoculated with diluted serum without virus and also uninoculated cultures. A rubella virus titration was included with every test series to check the titre of the rubella virus inoculum.

All groups of cultures were incubated at 35° C. for seven days. The maintenance medium was then removed and the cultures were washed twice with phosphate buffered saline, and the maintenance medium was then replaced. ECHO 11 virus (100 TCD_{50}) was then added to each test culture and to one-half of the cultures in the culture control group; the other half of this group remained as normal controls. All cultures were then incubated at 35° C. for two or three days until the cultures inoculated with ECHO 11 alone showed cytopathic changes affecting about two-thirds of each culture.

Before the results of a series of tests were considered acceptable, uninoculated culture controls had to be normal in appearance and serum control cultures were expected to react in a similar fashion to cultures inoculated with ECHO 11 alone. The rubella titration was checked to verify that the rubella inoculum did not vary by more than twice or less than half of the expected value of 100 InD_{50}.

RESULTS

The results of the serum titrations are given in Fig. 1, in which each point plotted represents the percentage of sera containing rubella antibodies in a group of 14 to 16 sera.

The data representing the percentage of persons with detectable antibody in their sera at a dilution of 1:6 show that by age 14 years 60% had antibodies and by 19 years 70% gave positive results.

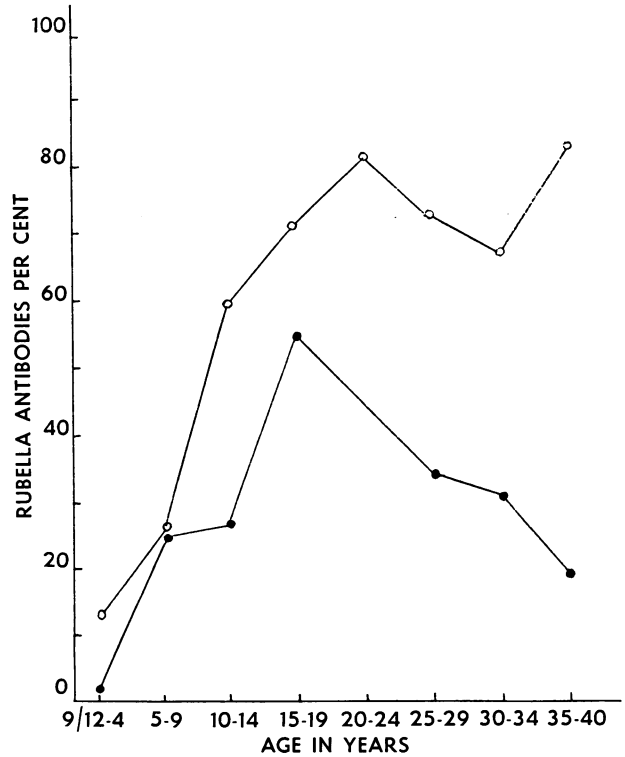

Fig. 1.—The incidence of rubella virus antibodies measurable at a serum dilution of 1:6 (upper curve) and 1:24 (lower curve). Each point represents an average incidence in a group of 14 to 16 female subjects.

This positive percentage increased to 80% by 24 years, but did not increase in the age groups above 24 years.

The percentage of women with antibodies detectable in the sera at a dilution of 1:24 is also plotted in Fig. 1. This curve represents the percentage of persons in each age group having a higher level of antibody.

The curves representing sera at both dilutions parallel one another until 19 years of age. This indicates that a constant proportion of individuals up to this age had high-level antibodies, and therefore it is presumed that a large number of individuals in groups between 1 and 19 years old had been infected recently with rubella virus. After 20 to 24 years, however, the curves diverge and the proportion of each age group with high level antibodies indicating recent infection declined progressively, until by 35 to 40 years only 20% had antibodies measurable at a 1:24 dilution of serum.

The test we have used seems to compare favourably with others that have been suggested. Schiff, Sever and Huebner[7] measured antibody in lots of commercial gamma globulin and obtained titres between $10^{-2.4}$ and $10^{-3.3}$. In our experience similar commercial lots had titres between $10^{-2.2}$ and $10^{-2.4}$. A test such as we have used is sufficiently sensitive for a serum survey, although it is expected that a method involving the inhibition of the direct cytopathic effect will shortly supersede the interference-inhibition type of test.

Canad. Med. Ass. J.
Jan. 16, 1965, vol. 92

DISCUSSION

Clinical experience suggests that 30-40% of women of child-bearing age (15-40) have a history of rubella with rash. An examination of Fig. 1 indicates that in these age groups between 60 and 80% of sera contain antibody, tested at 1:6 dilution. Obviously, therefore, a large number of infections must have pursued a subclinical course. That this is indeed the case has been demonstrated by Buescher[4] and co-workers by studies in army personnel.

It is also apparent from Fig. 1 that at least 20% of females in the child-bearing age group have no rubella antibody. We can expect, therefore, that these women run the risk of being infected with rubella virus on contact with a source of infection. If the exposed individual is pregnant when infected, there is the possibility that the virus may cause abortion, stillbirth, or congenital abnormalities. This risk has been estimated at 20-25% if infection occurs in the first trimester,[5] but this probability depends in part on physiologic and other undetermined factors in addition to virus infection.

In the group of females surveyed, the percentage of susceptibles, as judged by absence of antibody, decreased from 40% at 10 years of age to 20% at 24 years of age and remained at this figure until 40 years of age, the oldest group tested. This is in agreement with the work of Sever, Schiff and Huebner,[8] who established that the susceptibles in a group of 600 pregnant women, average age 25 years, numbered about 17%.

If a vaccine is to be used to prevent the various effects of rubella on the fetus, it would seem advisable to administer it during the middle and late teens so as to eliminate or much reduce this percentage of susceptibles. It would appear that nature carries on an extremely effective "silent" immunization program, but additional effort is required to convert to seropositive the residuum of susceptibles amounting to about 20% of the female population of child-bearing age.

A final observation may be made from Fig. 1. The figure shows that the percentage of females with high-titre antibodies falls off rapidly after 25 years of age, until by 40 years only 20% of the individuals are in this group. This is suggestive of a herd immunity waning with age. It is possible that falling antibody levels in the older groups may culminate in some individuals becoming resusceptible to rubella infection. If this is the case, then second attacks of a modified rubella may not be unusual and should not be overlooked because of a history of typical rubella earlier in life.

The authors wish to thank Mrs. Mary Frances Zeldman for her conscientious and able assistance.

REFERENCES

1. PARKMAN, P. D., BUESCHER, E. L. AND ARTENSTEIN, M. S.: *Proc. Soc. Exp. Biol. Med.*, 111: 225, 1962.
2. WELLER, T. H. AND NEVA, F. A.: *Ibid.*, 111: 215, 1962.
3. SIGURDARDOTTIR, B. *et al.*: *Canad. Med. Ass. J.*, 88: 128, 1963.
4. BUESCHER, E. L.: Personal communication.
5. MANSON, M. M., LOGAN, W. P. D. AND LOY, R. M.: Rubella and other virus infections during pregnancy, Ministry of Health, Reports on Public Health and Medical Subjects No. 101, Her Majesty's Stationery Office, London, 1960.
6. MCCARTHY, K., TAYLOR-ROBINSON, C. H. AND PILLINGER, S. E.: *Lancet*, 2: 593, 1963.
7. SCHIFF, G. M., SEVER, J. L. AND HUEBNER, R. J.: *Science*, 142: 58, 1963.
8. SEVER, J. L., SCHIFF, G. M. AND HUEBNER, R. J.: *Obstet. Gynec.*, 23: 153, 1964.

PAGES OUT OF THE PAST: FROM THE JOURNAL OF FIFTY YEARS AGO

DRAINAGE OF ABDOMINAL CAVITY

And this brings me to say a word about drainage of the abdominal cavity. When should we drain? It all depends upon the condition of the peritoneum. If it looks a good colour, is glistening, remove the gross soiling and close up. If it is very red and of a dull colour you must drain, preferably using a Mikulicz vaginal drain in the female. But don't forget that any drain in the general cavity becomes blocked off in a few hours and collections of fluid may form around it and can't drain into it. But when in doubt I say don't drain. It is wonderful, if your after-treatment is efficient, how much filth the peritoneum will take care of. But that is a big "if," and I am quite convinced that more lives are lost in cases of acute abdominal infections from inefficient post-operative measures than from inefficient operations.

Will you allow me to state just here our routine post-operative treatment of a case of general peritonitis, with or without drainage. The operation is finished with the patient in the Fowler position, and without changing that position (except perhaps slightly to exaggerate it) the patient is so put in bed. We thus keep our septic material in the basement. Our next object is to dilute this, and this we do by saline proctoclysis (Murphy), or by repeated (every four hours) large enemas of warm normal saline solution. Next, to eliminate the poison we depend upon nature's drain—the colon and rectum. Every four hours, alternating with the salines, the patient receives a soap-suds enema, to which is added one drachm of spirits of turpentine and twenty grains of quinine bisulphate. Nothing is given by mouth until the enemas are effectual and there is evidence of returning peristalsis, shown by passing flatus. Should the enemas prove ineffectual and abdominal distension, probably associated with vomiting, come on, we resort to the use of eserine, giving, hypodermically, one-sixtieth grain (combined with one-thirtieth of strychnine sulphate) every three hours for four doses. This in bad cases may be repeated the following day. But if the Fowler position has its great advantages, it always needs watching. Sometimes the vomiting comes on early and is explosive in character, like a case of high obstruction. And so it is. The Fowler position must then be changed to the reverse, or Trendelenberg, position, and the vomiting speedily stops. What has happened here? I have always noticed this accident to happen in fat persons, with a fat omentum, and my own theory is that this heavy and infected, omentum drags down upon the colon and stomach, when the patient is kept sitting up, sufficiently to produce an obstructive kink at the duodeno-jejunal fold. Reverse the position and the kink disappears, and so do the symptoms of high obstruction.—J. M. Elder, *Canad. Med. Ass. J.*, 5: 90, 1915.