fections. Each gibbon received 6.3 × 10^7 to 9.9 × 10^8 trophozoites by the intraperitoneal or the intravenous route. After a prepatent period of 2 to 20 days all animals had small ring forms in their erythrocytes (Table 1). These were followed by the larger trophozoites characteristic of falciparum malaria infections. The number of trophozoites increased rapidly and peaks were attained 1 to 5 weeks after the infections became patent. Asexual parasites were in the peripheral blood of most animals throughout the observation period but declined to approximately 1000 per cubic millimeter 2 to 3 weeks after reaching the height of parasitemia. All animals except P-11 had gametocytes in the peripheral circulation 4 to 25 days after inoculation. Peaks in the number of gametocytes usually paralleled the equivalent number of trophozoites but were of lesser magnitude. Most gibbons had approximately 1000 gametocytes per cubic millimeter of blood at varying intervals. P-1, with 9500 gametocytes per cubic millimeter of blood, had the highest number of sexual parasites in this series. In virtually all instances the gametocytes were immature. This was similar to the development of the parasite in the chimpanzee.

Intravenous inoculations of 2.65 × 10^7 trophozoites were made from gibbon P-7 to splenectomized (P-2 and S-2) and normal (P-12) subjects. The splenectomized gibbons developed definite parasitemias and P-2 had higher parasite counts than any other animal studied in these experiments. The gibbon with intact spleen (P-12) had a transitory parasitemia.

Most animals had a slight rise in rectal temperature associated with or just prior to the onset of parasitemia. Normal body temperature for these animals is approximately 101.5°F (38.6°C) while during the "fever" period it reached 103.5° to 104.5°F (39.7° to 40.3°C). The animals had a normal appetite and were alert during the observation period. White blood counts were made twice weekly but no appreciable fluctuations were evident.

The relative ease of infecting the splenectomized gibbon with blood-induced infections of *P. falciparum* indicates that this animal may be a suitable laboratory model for immunologic and chemotherapeutic studies of falciparum malaria. The development of an animal test system for this parasite may accelerate the study of drug-resistant strains *P. falciparum*, which has been hampered by the inherent danger of studying these parasites in nonimmune human volunteers (*4*, *5*).

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3. Animals received 2.5 mg chloroquine base per kilogram of body weight for 7 days and 0.75 mg primaquine base per kilogram of body weight for 14 days, 3 to 6 weeks prior to infection. Drugs were administered intramuscularly once daily.
4. *World Health Organ. Tech. Rep. Ser. 296* (1965).
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Nucleic Acid and Protein Changes in Wheat Leaf Nuclei during Rust Infection

Abstract. *Microspectrophotometric measurements of DNA, RNA, histone, and total protein in the nuclei of mesophyll cells of Little Club wheat leaves were made at intervals after infection with the stem rust fungus. No change in host DNA was found within 6 days after inoculation, but there were decreases in histones and marked increases in RNA and acidic proteins as early as 2 days after inoculation.*

Modern work, especially that of Flor (*1*), has led to the view that gene-for-gene relations underlie physiological specialization in plant pathogens such as the rust fungi, although we have virtually no knowledge of the specific biochemical processes involved. The effects of infection on the structure and nucleic acid content of the host nuclei are therefore of critical interest. It has long been considered that there is often a close association between the haustoria of the rust fungi and the nuclei of invaded host cells; the enlargement and subsequent collapse of the host nuclei in rust-infected cereal leaves were first documented over 40 years ago (*2*). More recently, Person (*3*) reported that the nucleoli in the enlarged nuclei in rust-infected wheat-leaf cells stained particularly strongly for RNA. Using a microspectrophotometer, Whitney *et al.* (*4*) measured the DNA (Feulgen) and RNA (azure B) in the nuclei of mesophyll cells of rust-infected leaves of Little Club, a susceptible variety of wheat. They found no changes in DNA content until the invaded cells senesced and the nuclei collapsed, but reported a doubling of the RNA content in the enlarged host nuclei associated with infections 6 days old.

Uredospores of *Puccinia graminis tritici* Erikss. and Henn., Race 15B, were painted onto zones 1.0 to 1.5 cm long in the centers of primary leaves of seedlings of Little Club wheat, 10 days after planting. Two days after inoculation leaf segments were fixed in a mixture of ethanol and acetic acid (3 : 1) or neutral Formalin (4 percent), embedded in Paraplast, and sectioned at 10 μ. DNA (Feulgen), RNA (azure B), histone (fast green, *p*H 8.1), and total protein (fast green, *p*H 2.0) were measured in arbitrary units with the two-wavelength method (*5*). The two wavelengths chosen in each case were selected from absorption spectra of appropriately stained nuclei and are given in Tables 1 and 2. We used standard procedures to determine RNA (*6*), DNA (*7*), histones (*8*), and total protein (*9*). Sections to be stained with azure B were first treated with deoxyribonuclease to remove DNA. Staining with fast green was carried out after removal of DNA and RNA with hot 5 percent trichloroacetic acid. Fast green at *p*H 8.1 was not bound to the histones unless DNA was first removed with deoxyribonuclease. Histone staining was also prevented by extracting sections with 0.2*N* HCl before fixation. These two criteria have been used (*10*) to define histones in polytene chromosomes of *Drosophila*. Sections stained for histones were rehydrated and restained for total protein (*9*). This procedure permits measurement of histones and total proteins on the same individual nuclei. The difference between total protein and histone was termed acidic protein. The values for histone and for acidic and total protein are in arbitrary units and are not necessarily comparable in absolute

Table 1. DNA and RNA in nuclei of rust-infected wheat leaves. The leaves were fixed in a mixture of ethanol and acetic acid for 3 to 4 hours in series 1, and 6 to 8 hours in series 2. DNA was measured at wavelengths of 490 and 511 mμ, and RNA at wavelengths of 510 and 536 mμ. The values for nuclear volume of host cells are means, and for DNA the values are means and standard errors; both are based on 15 to 20 nuclei, representing two or three leaves.

Days and status*	r† (μ^3)	Nucleic acid concentration (arbitrary units)	
		DNA	RNA
	Series 1		
2,H	34	6.5±.18	3.2±.53
2,R	41	6.6±.27	4.5±.49
4,H	41	6.1±.10	2.3±.18
4,R	51	6.3±.13	3.8±.71
6,H	36	6.4±.21	2.7±.28
6,R	88	6.6±.22	5.0±.07
	Series 2		
6,H	40	7.1±.26	2.7±.27
6,R	60	7.1±.26	7.7±.68
9,H	40	7.1±.05	2.7±.47
9,R	56	6.5±.24	7.0±.47
12,H	36	7.0±.13	2.8±.46
12,R	87	5.0±.13	6.7±.13
15,H	36	7.2±.31	2.7±.25
15,R		3.5±.23	5.2±.41

* After inoculation and treatment. H, uninfected; R, rust-infected. † Relative volume of host nucleus.

terms because the affinity of amino groups on the protein for the dye is not likely to be the same at both high and low *p*H values.

To determine the effects of infection, measurements were made on host nuclei situated near the centers of rust pustules. In the earlier stages of infection, at 2 and 4 days after inoculation, measurements were made on nuclei in mesophyll cells in contact with the fungal hyphae. Control measurements were made on nuclei in uninfected segments of infected leaves about 4 mm above and below the infected zones. The DNA content of mesophyll cell nuclei was equivalent to that of telophase (2C) nuclei in root tips of wheat.

Table 1 gives the results of two series of determinations of DNA and RNA in infected and uninfected leaves. There was no appreciable change in DNA until 12 days after inoculation, when the infected nuclei, then at their maximum recorded size, had lost 29 percent of their DNA. At 15 days, 51 percent of the DNA was lost. This result agrees well with the figure of 60 percent reported earlier for infections of the same age (*4*). Loss of DNA is associated with the senescence and death of invaded cells. It is important that during the first 9 to 10 days after infection the DNA content per nucleus was essentially constant. The RNA increased as early as 2 days after inoculation, reached a maximum at 6 days, and then declined slowly. Appropriate measurements on sections only 4 μ thick showed that both nucleolar and extra-nucleolar RNA in the nucleus increased after infection. These results for RNA fully confirm and extend those reported earlier (*4*).

The results in Table 2 for two further series of determinations again show that the amount of DNA in infected nuclei was not appreciably affected until a late stage (10th day) of rust development. In series 3, histones decreased by 35 percent, total proteins increased by 30 percent, and acidic proteins increased by 59 percent as early as 2 days after the inoculation. By the 6th day, histones had decreased by 50 percent, total proteins had increased by 69 percent, and acidic proteins by 123 percent, yet there was still no appreciable change in DNA. The results for the sixth day in series 4 are similar but less startling. Thus an early effect of infection with the rust fungus is to increase the ratio of DNA to histone and to increase nuclear RNA and total protein in the nuclei of host cells situated within the limits of rust pustules.

Increases in the size of host nuclei do not occur beyond the limits of the spread of the mycelium in rust-infected wheat leaves (*4*). The question of whether the presence of a haustorium within a cell is a prerequisite for the nuclear changes has not been satisfactorily resolved because of the difficulty of establishing with certainty the absence of haustoria from any particular cell in sections only 10 μ thick. Haustoria were often, but not always, present in the host cells in which the nuclei were measured. When present, a haustorium was sometimes in contact with the nucleus. In many cells the apparent absence of haustoria was clearly the result of sectioning at 10 μ. We believe that the presence of a haustorium in any particular host cell is not essential to the nuclear changes. The point could be much more easily and satisfactorily resolved by observations on leaves infected with powdery mildew since the mildew is an ectoparasite and does not penetrate the mesophyll of its host.

The significance of parasitically induced increases in RNA in host nuclei has been briefly discussed in earlier papers (*4*, *11*). Recent evidence from other systems indicates that histones play a key role in regulating nuclear function and that their removal in some way activates the synthesis of RNA (*12*). The decrease in histone and increases in RNA and acidic protein resulting from infection are all consistent with the view that a primary event in the infection process is the activation of particular genes in the nuclei of affected host cells. If this is so, the large early decrease in nuclear histones must involve an effect on a considerable proportion of the host DNA. The resulting alteration of specific metabolic pathways in the host may be assumed to determine the development of the rust fungus and the success

Table 2. DNA, histones, and total proteins in nuclei of rust-infected wheat leaves. The leaves were fixed in neutral Formalin (4 percent) for 3 to 4 hours in series 3 and 6 hours in series 4. DNA was measured at wavelengths of 490 and 511 mμ, and histones and total proteins at wavelengths of 580 and 615 mμ. Histones and total proteins were obtained for the same individual nuclei, and acidic proteins were obtained by taking the difference of the two. Values are means and standard errors for 8 to 12 nuclei, representing two or three leaves.

Days and status	r† (μ^3)	Concentration (arbitrary units)			
		DNA	Histones	Total proteins	Acidic proteins
		Series 3			
2,H	29.1	9.5 ±1.06	8.1± .39	26.3± .73	18.2
2,R	33.2	9.00± .59	5.3± .59	34.3±2.19	29.0
4,H	29.9	9.2 ± .48	7.9± .28	26.0± .45	18.1
4,R	34.6	9.3 ± .21	4.9± .43	35.5±2.81	30.6
6,H	28.4	9.1 ± .22	8.1± .46	26.1± .81	18.0
6,R	37.7	9.00± .20	4.1± .29	44.2±2.01	40.1
		Series 4			
6,H	31.1	9.2 ± .26	11.0± .51	21.0± .47	10.0
6,R	47.6	9.0 ± .12	7.1±1.54	25.3±1.12	18.2
10,H	29.7	9.1 ± .21	11.5± .19	20.2± .40	8.7
10,R	55.4	7.5 ± .40	6.4± .53	21.9± .74	15.5
15,H	29.9	8.6 ±1.07	12.3± .42	21.4± .75	9.1
15,R		5.3 ± .41	8.2± .94	19.9±1.54	11.7

* After inoculation and treatment. H, uninfected; R, rust-infected. † Relative volume of host nucleus.

or failure of the infection. The results for histones and total protein support the view that parasitically induced changes in host nuclei occur in the early stages of rust development and may lie at the core of this and other similar host-parasite relationships. This interpretation assumes that there is no significant change, as the infection progresses, of any masking effect of other groups on the dye-binding capacity of amino groups on the histones—that is, that the measured decreases in histones are real (*13*).

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Increased Activities of Glycogenolytic Enzymes in Liver after Splanchnic-Nerve Stimulation

Abstract. *Electrical stimulation of the splanchnic nerve of rabbits caused a marked increase, within 30 seconds after the onset of stimulation, in liver-glycogen phosphorylase and glucose-6-phosphatase activities. The increased activity of liver phosphorylase after splanchnic-nerve stimulation was likewise observed in adrenalectomized and pancreatectomized rabbits. Glycogen content of the liver decreased only slightly after 5-minute stimulation.*

Evidence has accumulated that the enzymatic composition of mammalian cells is not constant but can be altered by factors such as the type of nutrients, hormonal changes, and the administration of certain foreign chemical agents. Shimazu has reported that certain liver enzymes are influenced by the highest autonomic center of the hypothalamus (*1*), and has shown that the hypothalamic influence on the liver enzymes may be communicated through peripheral autonomic nerves (*2*).

We have demonstrated that the amounts of blood glucose and liver glycogen in rabbits are changed by electrical stimulation of the hypothalamus (*3*). Thus, electrical stimulation of the ventromedial hypothalamic nucleus (one of the nuclei in the sympathetic area of the hypothalamus) caused an increase in blood glucose followed by a pronounced decrease in liver glycogen, and electrical stimulation of the lateral hypothalamic nucleus (the parasympathetic area of the hypothalamus) caused a slight decrease in blood glucose with an insignificant change of liver glycogen.

We now report the effect of electrical stimulation of the splanchnic nerve, the peripheral sympathetic nerve innervating the liver, on glycogen phosphorylase and glucose-6-phosphatase activities of rabbit liver. The activities of these two enzymes were greatly increased within 30 seconds after the onset of stimulation of the splanchnic nerve. This result would properly explain the previous observations that blood glucose increases rapidly, whereas liver glycogen decreases markedly by hypothalamic stimulation of the sympathetic area (*3*).

Male rabbits weighing about 2300 g were lightly anesthetized with pentobarbital sodium (10 mg/kg, intravenously). Laparotomy was made under additional anesthesia with ether. The left splanchnic nerve was exposed and freed from surrounding adipose tissue just under the diaphragm, and a bipolar platinum electrode, fitted in a tiny plastic plate, was placed at the nerve. Fifteen to twenty minutes after placement of the electrode, one lobe of the liver was quickly removed by ligation and immediately immersed in liquid nitrogen. Stimuli delivered from an electronic stimulator (square pulses of 0.3 msec duration; frequency, 100 per second; amplitude, 50 volts) were applied to the splanchnic nerve through an isolation unit. After the indicated time of stimulation, the other lobe of the same liver was quickly removed and similarly frozen in liquid nitrogen while the stimulus was still being delivered.

For assay of phosphorylase, a portion (2 g) of each frozen liver was promptly pulverized and ground at −20°C with 2 volumes of 60-percent glycerol solution containing 0.05*M* NaF and 0.005*M* EDTA (ethylene diamine tetraacetic acid, adjusted with NaOH to *p*H 6.1) which had been chilled to near freezing state. The material was ground for about 10 minutes and then was diluted to 20 ml with cooled aqueous solution of the same salts. The suspension was centrifuged at 15,000*g* for 5 minutes at −5°C, and the supernatant was immediately analyzed for phosphorylase activity. The reaction mixture for assay contained 0.025*M* glucose-1-phosphate, 0.5 percent glycogen, 0.05*M* sodium-citrate buffer (*p*H 6.1), 0.025*M* NaF, 0.0025*M* EDTA (*p*H 6.1) and a suitable volume of the centrifuged liver extract in a total volume of 1 ml. Inorganic phosphate liberated during 5-

Table 1. Effect of electrical stimulations of the splanchnic nerve on phosphorylase and glucose-6-phosphatase activities, and gylcogen content of rabbit liver. S, stimulation; P_i, inorganic phosphate. Each row represents one rabbit.

Enzyme activity [mμmole (P_i) mg^{-1} (protein) min^{-1}]									
Phosphorylase				Glucose-6-phosphatase				Glycogen (mg/g of liver)	
Before S	After S			Before S	After S			Before S	After S
	30 sec	1 min	5 min		30 sec	1 min	5 min		
7.2		20.5	23.0	12.2		14.3	19.4	37.9	27.4
9.1		21.9	6.7	12.2		16.3	16.9		
4.3	16.1	9.5	23.9	15.1	21.5	21.9	18.0	26.3	21.4
5.5	20.0	11.7	24.5	19.4	24.0	28.3			
10.4	24.5	30.8	33.4	20.2	29.5	24.8	26.7	44.3	43.6
*P values					.02>P>.01	.02>P>.01	.02>P>.01		.2>P>.1

* The t-test was made by paired observations (after stimulation compared to before stimulation).