Table 1. Data on pyrite removal. All coals were washed prior to use. Each coal sample (100 g) was pulverized to −100 mesh top size for accurate sampling (no decrease in the amount of sulfur that can be removed for larger particle sizes has been observed). Each coal sample was treated with four 1-hour leaches with 1*M* $FeCl_3$; Fe_2SO_4 gives almost the same results as $FeCl_3$. The elemental sulfur was removed with hot toluene after aqueous treatment.

Coal*	Sulfur content (% by weight) of		Pyritic sulfur removed‡ (% by weight)	Total sulfur removed (% by weight)
	Coal as received†	Coal after treating†		
Number 6	3.81	2.19	98	43
Number 5	3.49	2.02	94	42
Pittsburgh	1.77	0.77	84	57
Lower Kittanning	4.30	1.13	88	74

* Sources: number 6, Randolph County, Illinois; number 5, Fulton County, Illinois; Pittsburgh, Greene County, Pennsylvania; Lower Kittanning, Indiana County, Pennsylvania. † Total sulfur content and sulfur forms (dry basis) were determined by American Society for Testing Materials techniques at the Commercial Testing and Engineering Laboratories, Chicago, Illinois. ‡ We confirmed the values for the amounts of sulfur removed by analyzing the aqueous extract for its sulfate content and the toluene extract for its elemental sulfur content.

Some typical experimental results (average of duplicate runs) are shown in Table 1 for four major U.S. coals. It can be seen that for these four coals almost all of the pyritic sulfur is removed, while the decrease in the total amount of sulfur varies from 42 to 74 percent because of the varying organic sulfur content. The molar ratio of sulfate to sulfur formed as a result of the treatment with ferric salt varies from 0.2 to 2.5. The heat content of the coal increases and the ash content decreases as a result of pyrite removal.

These four coals were selected for evaluation because their distribution of sulfur forms is typical of coals east of the Mississippi River and because they represent major U.S. coal beds. The Pittsburgh bed has been described as the most valuable individual mineral deposit in the United States and perhaps in the world. Its production accounts for approximately 35 percent of the total cumulative production of the Appalachian bituminous coal basin up to 1 January 1965, and 21 percent of the total cumulative production of the United States to that date (*6*). The Lower Kittanning bed together with its correlative beds contain even larger reserves than the Pittsburgh seam. The number 5 bed is the most widespread and commercially valuable coal bed in the eastern interior coal basin. The Herrin number 6 bed is second in commercial importance only to the number 5 bed. Thus, the experimental results presented here indicate that the sulfur content of a significant portion of U.S. coal can be lowered by at least 40 to 75 percent if the coal is treated with aqueous ferric solution.

A block diagram of one preliminary process design utilizing the technical approach described above for the removal of pyritic sulfur from coal, together with an air oxidation regeneration scheme for the ferric leach solution, is shown in Fig. 1.

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References and Notes

1. "Air Quality Criteria for Sulfur Oxides" [U.S. Department of Health, Education, and Welfare National Air Pollution Control Administration Publication No. AP-50 (January 1969)].
2. We have found that ferric sulfate and ferric chloride are most effective.
3. The use of aqueous ferric salts to oxidize and dissolve copper sulfide ores is well known [for example, F. P. Haver and M. M. Wong, *J. Met.* **23** (No. 2), 25 (1971)]; however, Haver and Wong state (without direct proof) that iron pyrite in copper ore concentrates is not attacked in a period of hours at 100°C. In much earlier work, it was variously claimed (i) that ferric salts do not attack pyrite or (ii) that the reaction has a 60 to 80 percent level of completion [J. W. Mellor, Ed., *A Comprehensive Treatise on Inorganic and Theoretical Chemistry* (Wiley, New York, 1961), vol. 14, pp. 231–232]. In contrast, we have found that treatment of −200 mesh top size iron pyrite mineral with 1*M* aqueous ferric chloride solution at 100°C results in 49 percent dissolution after 2 hours, 96 percent after 8 hours, and 99.5 percent after 16 hours.
4. It was not obvious at the start of our work that elemental sulfur could be removed from the coal matrix; earlier reports had indicated that coal heated with elemental sulfur resulted in recombination and elimination of hydrogen sulfide [for example, B. K. Mazumdor, *Fuel* **41**, 121 (1962)].
5. The literature contains a number of references to the air oxidation of ferrous salts [for example, E. J. Sercombe and J. K. Gary, British Patent 1,143,139 (1969); V. V. Ermilov, Y. P. Romanteev, Yu. A. Shchurouskii, *Tr. Inst. Met. Obogashch. Akad. Nauk Kaz. SSR* **30**, 55 (1969); L. Liepina and B. Macejevskis, *Dokl. Akad. Nauk SSSR* **173** (No. 6), 1336 (1967)].
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7. We thank T. K. Janes, L. Lorenzi, and L. Cramer of the Environmental Protection Agency and J. Blumenthal, E. R. Boller, E. A. Burns, B. Dubrow, W. Krawitz, A. A. Lee, and L. J. Van Nice of TRW Systems for their encouragement. Presented in part by R.A.M. at the Symposium on the Desulfurization of Coal, 71st national meeting of the American Institute of Chemical Engineers, Dallas, Texas, 22 February 1972, and at the All-Union Session on Coal Conversion and the Environment, American Geophysical Union, Washington, D.C., 19 April 1972. Supported by the Environmental Protection Agency (after initial feasibility demonstration) under contract EHSD 71-7.

31 March 1972; revised 23 June 1972 ■

Absence of Polymerase Protein in Virions of Alpha-Type Rous Sarcoma Virus

Abstract. *Noninfectious particles of a mutant of Rous sarcoma virus failed to exhibit DNA polymerase activity even with the use of the most sensitive synthetic template-primer complexes. A neutralization blocking test against antibody to DNA polymerase revealed that these mutants did not contain protein immunologically related to the DNA polymerase.*

A small fraction of clones of chicken cells transformed by the Bryan strain of Rous sarcoma virus (RSV) is known to produce only noninfectious virus particles, whereas the majority produce infectious RSV (*1*). The defect of this noninfectious virus, called RSVα, can be complemented by helper leukosis virus but not by the viral genome existing in normal chicken cells (*1*, *2*). Characterization of the RSVα particles demonstrated that these are deficient in RNA-dependent DNA polymerase that is generally found in the virions of RNA-containing oncogenic viruses (*3*). Independent work by Robinson and Robinson (*4*), however, suggested that this type of RSV has an abnormal enzyme that is inactive with the endogenous viral RNA as template, but active with exogenous DNA templates. Moreover, since the original workers (*3*) did not use synthetic templates that can substantially heighten the sensitivity of the enzyme assay (*5*, *6*), the existence of a low level of enzyme in RSVα could not be ruled out.

In this study, we first examined the enzyme activity in RSVα particles with the most sensitive assay systems available. Then we asked whether or not the enzyme protein is present in a functionally inactive form by examining possible reaction with a monospecific

Table 1. DNA polymerase activity of RAV-2 and RSVα. DNA polymerase was assayed by a method essentially similar to that described (*5*). Samples were incubated for 60 minutes at 37°C. Reaction mixtures for (A) contained 80 pmole of [^{3}H]dTTP (30,000 count/min per picomole), 100 nmole of dATP, 100 nmole of dCTP, and 95 nmole of dGTP in 0.2 ml. Reaction mixtures for (B) contained 40 pmole of [^{3}H]dTTP (30,000 count/min per picomole), 3000 pmole of poly(A) and 300 pmole of $(dT)_{12\text{-}18}$. A background of 0.017 pmole was found for an unincubated control sample and was subtracted from each experimental sample.

Virus	Amount (μg)	[^{3}H]dTMP incorporated (pmole)
	(A) Endogenous	
RAV-2	0.35	0.03
	35	5.10
RSVα	35	<0.01
	(B) Poly(A) · oligo(dT) as a template	
RAV-2	0.01	0.07
	0.07	0.39
	0.14	0.76
	0.28	1.75
RSVα	1	<0.01
	2.5	<0.01

antiserum against avian myeloblastosis virus polymerase.

The alpha-type RSV released from transformed cells will be simply called RSVα in this report. These particles correspond to RSVα (0) or RSVα (f), and their preparation has been described. Two preparations obtained from transformed cells derived from single foci were used. Avian leukosis viruses, avian myeloblastosis virus (AMV) and RAV-2, and the sarcoma virus, Schmidt-Ruppin RSV (SR-RSV), and Bryan wild-type RSVβ (f) and RSVβ (—) were also used.

The endogenous DNA polymerase activity in preparations of RSVα was first compared with that of RAV-2, a completely active avian leukovirus. When 35 μg of viral protein from each stock was compared, no activity was demonstrable in the RSVα stock, whereas extensive incorporation was observed with the RAV-2 stock (Table 1). Even a 100-fold dilution of the RAV-2 stock gave detectable activity (threefold over background of unincubated sample), indicating that RSVα contains less than 1 percent of the endogenous DNA polymerase activity of RAV-2.

A number of different template-primer combinations have been tested with both RAV-2 and RSVα. When poly(A) · oligo(dT) was used, extensive incorporation with RAV-2 was observed while RSVα gave no significant amount of incorporation (Table 1). Incorporation with 2.5 μg of RSVα protein was insignificant, while 0.01 μg of RAV-2 protein gave incorporation tenfold higher than background. Thus, when poly(A) · oligo(dT) is used (*7*), RSVα has less than 0.1 percent the activity of RAV-2.

Stocks of RAV-2 and RSVα, and a mixture of the two, were assayed for activity with poly(C) · oligo(dG), as well as with poly(A) · oligo(dT). It is evident from Table 2 that RSVα exhibited little detectable activity with either template. With both templates, the mixture of the two stocks showed the full activity of RAV-2 alone, indicating that no inhibitor was present in the RSVα stock.

When a preparation of activated DNA (*8*) was assayed as a template-primer for DNA synthesis, RAV-2 again showed extensive incorporation, as did a preparation of SR-RSV, while RSVα showed no detectable activity (Table 3). The limit of detection in this assay was about 1 percent of the RAV-2 or SR-RSV activity.

Using poly(A) · poly(dT) as a template, we also compared the enzyme activity of RSVα with that of two forms of Bryan RSVβ, and of AMV. As shown in Table 4, the two forms of Bryan RSVβ had almost the same

Table 2. Poly(A) · oligo(dT) and poly(C) · oligo(dG) as templates for DNA synthesis with RAV-2 and RSVα. Samples were incubated for 60 minutes at 37°C. Reaction mixtures for [^{3}H]dTMP incorporation were the same as Table 1. For dGMP incorporation, 0.1 ml reaction mixtures contained 185 pmole of [^{3}H]dGTP (7000 count/min per picomole), 2600 pmole of poly(C), and 270 pmole of $(dG)_{18\text{-}12}$.

Virus	Amount (μg)	[^{3}H]-dTMP incorporated (pmole)	[^{3}H]-dGMP incorporated (pmole)
RAV-2	0.14	0.83	40.48
RSVα	1	<0.01	0.01
RAV-2 + RSVα	0.14 + 1	0.90	35.42

specific activity as AMV, while no significant activity was demonstrable with RSVα. The RSVβ (—) is another noninfectious virus, released from transformed cells without any assistance of helper virus or helper factor. The positive enzyme activity in RSVβ(—) confirms the notion that the defect of this form of RSV is not in the polymerase (*3*). We conclude from these results that preparations of RSVα contain no detectable DNA polymerase activity either with endogenous template or with various exogenous templates.

In the next experiments, we con-

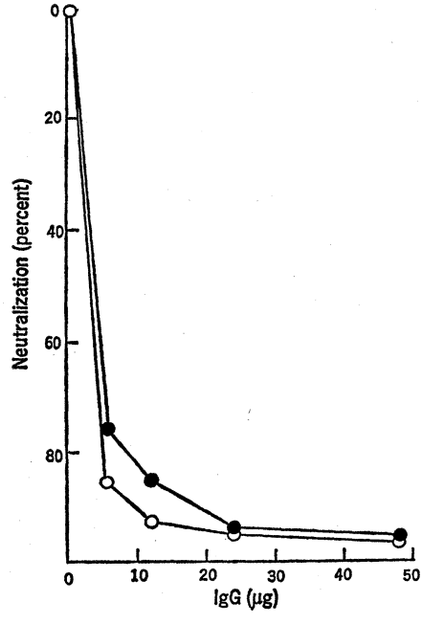

Fig. 1. Neutralization of Bryan RSVβ-DNA polymerase activity by IgG from antiserum to AMV DNA polymerase. Purified virus suspensions were disrupted for 30 minutes at 0°C with 0.2 percent Nonidet-P40 detergent. In a volume of 60 μl, the virus suspension was mixed with increasing amounts of IgG protein from antiserum to AMV DNA polymerase. The IgG protein fraction from normal rat serum was used as a control. The reaction mixture also contained 0.01*M* tris-HCl, *p*H 8.2, 0.15*M* potassium chloride, 20 percent (by volume) glycerol and 50 μg of bovine serum albumin. After 15 minutes at 37°C, standard polymerase assay conditions were established by addition of triphosphate and templates (Table 4). The acid-precipitable radioactivity represents the DNA polymerase activity not neutralized by antibody against AMV DNA polymerase. To establish how much viral enzyme from RSVβ(f) and RSVβ(—) disrupted by detergent was required for the neutralization tests, an enzyme activity was titrated against increasing amounts of viral protein. With AMV virion enzyme activity as a reference, one unit of each viral enzyme activity was added per assay. The one unit of AMV virion polymerase activity represents the amount of detergent-disrupted virion suspension necessary to render acid-precipitable 1 pmole of [^{3}H]TTP per minute with poly(dT) · poly(rA) as a template in a standard polymerase assay. The rate of incorporation of [^{3}H]TMP at this concentration of viral enzyme activity was linear for more than 90 minutes.

Table 3. "Activated" DNA as a template for DNA synthesis with RAV-2, SR-RSV, and RSVα. Samples were incubated for 60 minutes at 37°C. Standard 0.1 ml reaction mixtures were prepared as described for (A) in Table 1. Where indicated, 13 nmole of "activated" calf thymus DNA (*8*) was added.

Virus	Amount (μg)	[^{3}H]dTMP incorporated	
		Endogenous (pmole)	+ DNA (pmole)
RAV-2	0.14	< 0.01	0.46
	0.7	0.06	2.14
	1.8	0.17	4.79
SR-RSV	0.6	0.04	7.84
RSVα	2	0.01	< 0.01

ducted a collaborative study with R. C. Nowinski (McArdle Laboratory, University of Wisconsin) to determine if RSVα contains immunologically active, but nonfunctional polymerase protein. For these studies, detergent-disrupted RSVα was tested for its ability to absorb antibody from a monospecific antiserum prepared in rats against purified AMV polymerase (*9–11*). The antiserum used in these tests has been previously shown to be active against a variety of avian leukosis and sarcoma viruses (*9*, *10*), and as illustrated in Fig. 1, it was also active against DNA polymerase of two types of Bryan RSVβ.

The IgG from antiserum to AMV DNA polymerase was mixed with a virus suspension that had been previously disrupted with 0.2 percent Nonidet-P40 for 30 minutes at 0°C. This mixture of disrupted virus and antibody was then further incubated at 37°C for 60 minutes. This condition was satisfactory to inactivate the original viral enzyme so that the residual antibody activity could be measured by addition of one unit of fresh viral enzyme to the mixture. Figure 2, A and B, shows that the antibody was almost completely blocked by preliminary incubation with AMV, since no antibody activity could be detected when additional AMV polymerase was added to the reaction mixture. However, when the antibody was first incubated in the same manner with approximately a tenfold greater amount of RSVα-disrupted virions, virtually no reduction in antibody activity for AMV-polymerase was demonstrated. These results indicate that RSVα virions are deficient in DNA polymerase even as an immunologically detectable protein; the results are in agreement with another observation (*10*).

Recently, Weissbach *et al.* (*12*) found

Table 4. Comparison of DNA polymerase activity of AMV, RSVβ(f), RSVβ(—), and RSVα. The assay of the polymerase was performed by the method described previously (*14*). Samples were incubated for 60 minutes at 37°C. Reaction mixtures (0.1 ml) contained 10.5 nmole of [^{3}H]dTTP (300 count/min per picomole), poly(A)·poly(dT), 1.2 nmole of polymer phosphate per strand, and 20 nmole of dATP. Viruses were disrupted with 0.2 percent Nonidet-P40 for 30 minutes at 0°C.

Virus	Amount (μg)	[^{3}H]dTMP incorporated (pmole)
AMV	0.1	29
	0.2	64
	0.3	111
RSVβ(f)	0.5	136
	1.0	271
RSVβ(—)	0.35	85
	0.7	153
RSVα	18	< 0.55

no tumor virus-specific RNA-dependent DNA polymerase in chicken cells transformed by RSVα type. Therefore, it seems likely that RSVα is either a deletion mutant for the polymerase gene, or is deficient in an RNA subunit which contains the polymerase locus. However, the possibility still remains that RSVα might contain inactive protein which does not cross-react im-

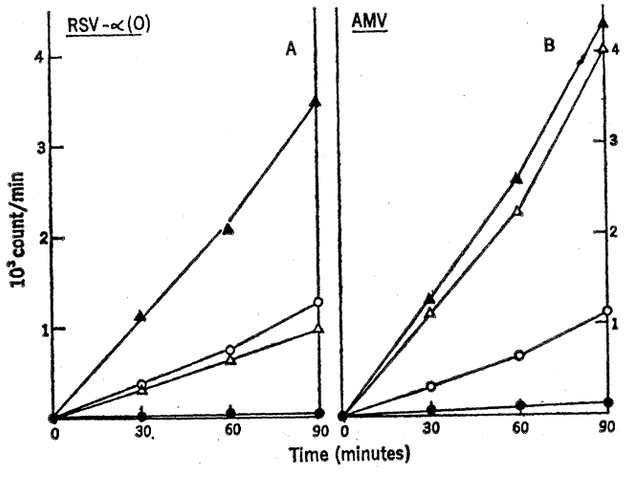

Fig. 2. (A) Failure of RSVα to block neutralization of AMV DNA polymerase by the IgG fraction of antiserum to AMV DNA polymerase. (B) Ability of AMV to block neutralization of AMV DNA polymerase with the same IgG. In a reaction mixture of 50 μl containing 0.01*M* tris-HCl, *p*H 8.2, and 0.15*M* potassium chloride, 3 μg of the IgG fraction from antiserum to AMV DNA polymerase was mixed with a given amount of test virus suspension that had been previously disrupted with 0.2 percent Nonidet-P40 for 30 minutes at 0°C. After 60 minutes at 37°C (incubation 1), glycerol (final concentration 20 percent), and bovine serum albumin (50 μg) were added to the reaction mixture, followed by the addition of one unit (see Fig. 1) of detergent-disrupted AMV virion DNA polymerase activity. This mixture (70 μl) was then incubated at 37°C for 60 minutes (incubation 2). To complete the assay, the volume of the mixture was increased to 110 μl with the addition of standard polymerase assay components as described (Fig. 1). Upon incubation at 37°C, 25-μl portions were removed at 0, 30, 60, and 90 minutes for determination of acid precipitable radioactivity. Residual antibody after incubation 1 was measured by its ability to neutralize AMV virion enzyme activity in incubation 2. To evaluate inhibition of neutralization, reaction mixtures were prepared as follows: (●—●) Test virus suspension and normal rat IgG in incubation 1 with no addition of AMV virion enzyme for incubation 2. This control shows that the test virus suspension DNA polymerase activity is inactivated during incubation 1 and is, therefore, not contributing activity in the final polymerase assay. (○—○) IgG from antiserum to AMV DNA polymerase in incubation 1 with one unit of AMV virion enzyme activity added for incubation 2. This control establishes the level of neutralization by the given amount of the IgG from antiserum to AMV DNA polymerase. (▲—▲) Test virus suspension and normal rat IgG in incubation 1 with one unit of AMV virion enzyme activity added for incubation 2. This control shows the effect of test virus suspension and IgG protein in incubation 1 on the AMV virion enzyme activity added in incubation 2 and established the optimum value of activity to be reached by inhibition of neutralization. (△—△) Test virus suspension and IgG from antiserum to AMV DNA polymerase in incubation 1 with one unit of AMV virion enzyme activity added for incubation 2. Test virus suspension that is antigenically related to AMV virion DNA polymerase will absorb the IgG from antiserum to AMV DNA polymerase in incubation 1, reducing the amount available for neutralization of AMV virion enzyme in incubation 2. Incubation 1 contained 2 μg of AMV or 30 μg of RSVα viral protein.

munologically. In addition to RSVα, noninfectious forms of mouse sarcoma-leukemia virus have been reported to have very low levels of DNA polymerase (*13*). None of the infectious forms has been found deficient in this enzyme.

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6 July 1972; revised 12 August 1972

Polychlorinated Biphenyl Residues: Accumulation in Cayuga Lake Trout with Age

Abstract. *The concentration of polychlorinated biphenyls was shown to progressively increase with maturity in a series of lake trout. The presence of these compounds was determined by column chromatographic isolation, specific detector gas chromatography and mass spectrometry. The relation between fish age and the concentration of polychlorinated biphenyls was highly significant.*

Residues of polychlorinated biphenyls (PCB's) have been reported in fish (*1*), and general environmental contamination by these compounds has been reviewed (*2*). The concentration of DDT residues in a series of lake trout from Cayuga Lake in Ithaca, New York, was found to be proportional to their age (*3*). Since PCB's are similarly stable, fat-soluble compounds, analysis for them was performed on the same series of fish.

The fish were netted in October 1970. Their ages were accurately known, because the fish are annually stocked as yearlings and distinctly marked as to year class. Without evisceration, each fish was mechanically chopped, ground, and thoroughly mixed. Fish samples (5 g) were dried and extracted with hexane in a Soxhlet apparatus for 3 hours. The hexane extracts were concentrated, and PCB's were separated from DDT residues and other constituents by adaptations of the methods (*4*) involving sulfuric acid partitioning and column chromatography on silica gel. Final analysis was made by electron affinity gas chromatography with a column 30 cm long, consisting of 10 percent DC-200 on 100- to 120-mesh Gas-Chrom Q and operated at 185°C. The concentration was estimated by the method of Risebrough (*5*), in which the response of each PCB isomer is taken as equal to that of the corresponding weight of *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE). This method was sensitive to about 0.25 part per million (ppm) of PCB's in the fish. Analysis of PCB's in five portions of the same fish were 6.4, 6.0, 5.6, 5.6, and 5.3 ppm. The standard deviation for these analyses is 0.427.

Table 1 lists the concentrations of PCB's in the lake trout as a function of their age, length, and weight. The correlation coefficient for PCB concentration as a function of age was 0.86 and was highly significant. The correlation coefficients relating PCB concentration with length and weight for fish 2 years and older were 0.85 and 0.80, respectively. The weights and lengths of the year-old trout were not recorded. The relation between the concentration of PCB's in the lake trout and their age is shown in Fig. 1; the curve represents the best fit of the data and has the equation:

$$\mathrm{PCB} = 1.031\, e^{0.259a}$$

where a is age. Figure 2 shows gas chromatograms of PCB's in a 12-year-old lake trout and of Aroclor 1254 standard (a mixture of chlorinated biphenyls containing 54 percent chlorine) to illustrate the similar peak retention times. The relative peak heights of individual PCB isomers in the chromatogram did not vary with age of the fish. This would indicate that there is no selective metabolism or storage of specific PCB isomers as the fish mature. Combined gas chromatography–mass spectrometry was used to verify the presence of the various PCB isomers in a 12-year-old lake trout (PCB's, 26.2

Table 1. Residues of PCB's in Cayuga Lake trout as a function of maturity; J, juvenile.

Age (years)	Sex	Length (cm)	Weight (g)	PCB (ppm)
1	J			0.6
1	J			1.6
1	J			0.5
1	J			1.2
2	J	27.7	181	2.0
2	J	28.7	226	1.3
2	J	33.5	407	2.5
3	J	44.5	815	2.2
3	J	44.5	725	2.4
3	J	41.1	770	1.2
4	J	53.8	1310	3.5
4	J	50.3	1160	4.1
4	J	55.1	1359	5.1
5	M	61.0	2030	5.7
6	M	63.5	2440	3.4
6	M	66.4	2850	9.7
6	F	68.3	2310	8.6
7	M	63.5	2260	4.0
7	M	68.9	3300	5.5
7		59.7	1990	10.5
8	F	75.2	3390	17.5
8	M	71.6	2805	13.4
8	F	69.0	3300	4.5
9	F	71.2	3390	30.4
11	M	80.3	4200	12.4
12	M	71.6	2535	13.4
12	M	75.5	3120	26.2
12	F	70.6	3440	7.4